Castellani, (Sir) Aldo
Fungi and fungous diseases

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FUNGI

AND

FUNGOUS DISEASES

by

Aldo Castellani, C.M.G., M.D.

Lecture 1

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FUNGI AND FUNGOUS DISEASES

LECTURE I *

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NEW ORLEANS

FUNGI

Historical—Morphologic Characters of Fungi—Classification—Biochemical Activities of Fungi—Symbiosis—A Mycologic Method for the Identification of Various Sugars and Other Carbohydrates.

Mycology, the science that deals with the study and investigation of fungi *sensu stricto* or mycetes, in contrast to bacteriology, has attracted comparatively little notice in recent years. Scant attention has been paid to the enormously important rôle played in nature by fungi and to the evil they are capable of doing, as well as to the beneficent functions they may perform.

It is interesting to note, however, that mycology came into existence long before bacteriology. It may be said to have begun in the days of Charles II, when Hooke, in 1677, constructed a magnifying lens with which he examined the yellow spots so often present on the leaves of the damask rose, and found that they consisted of filamentous fungi of which he gave a detailed description and left remarkably good drawings. He believed that fungi arose from decaying matter by a process of spontaneous generation. Malpighi, in 1686, devoted a chapter to "Plantis quae in aliis vegetant," in which he refers to fungi of the genus *Mucedo*. Ray, in 1706, in his "Historia Plantarum" described *Pilobolus crystallinus,* one of the mucoraceae which has beautiful crystalline sporangia. Micheli, in 1729, gave an account of the genus *Aspergillus* and many other fungi. In 1752, Linnaeus, in his famous book "Species Plantarum," collected all that was known at the time on the subject, and named a large number of species. After Linnaeus, many botanists carried out investigations on fungi: it suffices to mention the names of Hudson, who described many mucors in his "Flora Anglica" (1762); Lightfoot, who published an important work on the Scottish flora in 1777; Pelham (1785); Withering (1795); Batsh (1783) in Germany, and Bulliard (1791) in France.

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* The Adolph Gehrmann Lectures of the University of Illinois College of Medicine, 1926.

At the beginning of the last century, the progress became rapid, and the only way to trace its evolution is to mention the great systematic works, which are used for reference. The principal ones are Persoon (1801) "Synopsis Methodica Fungorum"; Link (1824) "Caroli Linné Species Plantarum" (1824). Editio Quarto tomus VI, p. 1; Fries (1821-1829) "Systema Mycologicum"; Nees von Esenbeck and Henry (1837) "Das System der Pilze"; Kutzing (1849) "Species Algarum"; Charles Robin (1853) "Vegetaux Parasites"; Kucheinmeister (1857) "Animal and Vegetal Parasites of Man; Fuckel (1869-1870) "Symbolae Mycologiae"; Eidam (1872) "Mycologie"; Naegeli (1877) "Die Niederen Pilze."

In 1881, Winter, Rehm, Fischer and Lindau's "Die Pilze Deutschlands" began to appear, and in 1886, Saccardo's great work of reference, the "Sylloge Fungorum," began. Both have continued to the present period. In 1890, Zopf published his well known work, "Die Pilze"; in 1900, Engler and Prantl published the "Pflanzen familien"; in 1907, Vuillemin started his new classification in his paper, "Les bases actuelles de la systematique en mycologie." Pollacci and Nannizzi in 1924 started the publication of an exhaustive work on pathogenic fungi "Imiceti patogeni dell'Uomo e degli Animalin."

Parasitic fungi of man[1] did not attract attention until the beginning of the last century. The first important fungus discovered was the thrush-fungus, observed by Langenbeck in 1839. He discovered it at autopsy in a case of typhus while examining microscopically the white patches of thrush in the oral mucosa, the pharynx and the whole of the intestinal tract. He believed at first that the fungus was the cause not only of the white patches but also of the typhus infection from which the patient died. The knowledge of this fungus was greatly increased in 1843 by the investigations of Charles Robin, who called it *Oidium albicans*. Robin also wrote a book on mycology which has remained classic: "Histoire naturelle des vegetaux parasites qui croissent sur l'homme et sur les animaux vivants."[2] The idea that thrush was a mycologic condition was not generally accepted for a long time. Even a number of years after Robin's classic work, many physicians did not believe in it. In one of the most popular textbooks on pathology of the fifties and the sixties of last century, one reads that the thrush patches are the result of morbid secretion of the oral mucosa, and the author added: "A mycologist, however, has brought forward the very peculiar hypothesis that these patches are caused by a fungus." In the same year that the fungus of thrush was discovered by Lagenbeck, another important fungus was found: the fungus of favus, by Schönlein. This organism a little later was further investigated by Lebert, who called it *Oidium schönleinii,* and subsequently by Remack, who named it *Achorion schönleinii.* In 1844, the fungus of ringworm was found by Gruby, who also pointed out the important difference between ringworm fungi of the large spore type and of the small spore type, an idea which was ridiculed at the time, and completely forgotten later, until Sabouraud, many years after, made the same distinction and called attention to Gruby's forgotten work.

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[1] Medical mycology is quite extensively dealt with in Brumpt's "Précis de Parasitologie" and Castellani and Chalmer's "Manual of Tropical Medicine."

[2] Robin: Histoire naturelle des vegetaux parasites qui croissent sur l'homme et sur les animaux vivants, Paris, J. B. Baillière, 1853.

In 1846, Eichstedt discovered the fungus of pityriasis versicolor.

Mycology continued to attract attention until the seventies and eighties of last century, when the great discoveries by Pasteur and Koch in the field of bacteria brought bacteriology to the foreground, and mycology was placed in the background, where it has remained until the present. There can be little doubt, however, that in future years the great importance of mycology will be recognized; one has only to remember that more than 20 per cent of tropical diseases *sensu stricto* are caused by fungi, and that diseases due to fungi are also not at all rare in temperate climates.

GENERAL REMARKS

Morphologic Characters of Fungi. Classification.—As is well known, the *regnum vegetabile* is usually divided into four large groups or phyta:

1. *Spermatophyta,* or seed plants.
2. *Pteridophyta,* or fern plants.
3. *Bryophyta,* or moss plants.
4. *Thallophyta,* or thallus plants.

Thallophyta, to which the fungi belong, are generally filamentous plants, of simple cellular structure, which differ from other plants in that they do not develop a complex plant—body with differentiation into roots, stem and leaves.

Thallophyta are separated into two large subdivisions:

1. *Algae,* chlorophyl present.
2. *Fungaceae* (fungi *sensu lato*), chlorophyl absent.

Fungaceae or fungi *sensu lato* may be divided into two large groups:

(*a*) Bacteria, or lower fungi *(Schizomycetaceae)*, unicellular plants, of minute dimensions, which multiply usually by cell fission.

(*b*) Fungi *sensu stricto*, higher fungi, mycetes *(Mycetaceae)*, unicellular or pleuricellular plants, of larger dimensions than bacteria, which multiply by processes other than simple cell fission, usually by means of asexual or sexual spores.

At present the term "mycology" is limited to the study of the higher fungi or mycetes, while "bacteriology" deals with the lower fungi or bacteria.

Fungi, not possessing chlorophyl, are unable to build up their food from the carbon dioxide of the air, and must obtain it from larger vegetal organisms, from animals or from decaying matter. Fungi may be parasitic or saprophytic: parasitic fungi grow and obtain their food from living animals or living plants; saprophytic fungi grow and feed on dead plants or decaying matter in general. In medical literature, the term "saprophytic" is used to denote a fungus that is not pathogenic.

Some fungi are always saprophytic, others always parasitic; but there are also fungi which are facultative parasites—that is, they are saprophytic, but under certain conditions they become parasitic. The animal or plant on which the fungus grows is said to be the "host."

As in the case with the flowering plants, a fungus consists of (1) a vegetative part (thallus, mycelium) devoted to the acquisition of food; (2) a reproductive part. The vegetative part consists of fine filaments or threads called "hyphae"; a collection of hyphae is known as mycelium "vegetative part of the fungus." A hypha is a hollow cylindric tube containing protoplasm and divided at intervals by partitions called "septa." It may be of different shapes, straight, curved or banana-like. Hyphae may have peculiar morphologic characteristics of great importance for classification: for instance, the peculiar swellings on the hyphae of the *Borellineae* and the clamp connections of the *Basidiomycetae*.

Reproduction.—Fungi reproduce by means of specialized cells termed spores, which may in a way be said to represent the seeds of the flowering plants. A fungus spore is merely a spheric, oval or elongated cell with a thin wall, and containing protoplasm. A spore may multiply by budding, producing daughter spores identical with the parent spore; if a spore meets with favorable conditions, it may germinate by growing out a filament or hypha, which develops into a mycelium.

The spores belong to two types: those that are sexual in origin, and those that are asexual in origin. The asexual spores are those which are not the result of the fusion of two sex cells or gametes. Practically all fungi produce asexual spores; some produce both asexual and sexual spores.

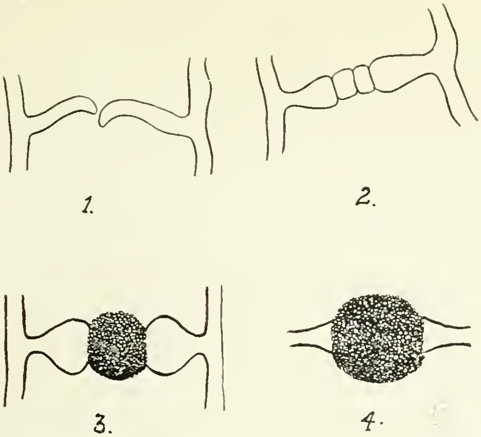


Fig. 1.—Various stages in the process of zygosporosis.

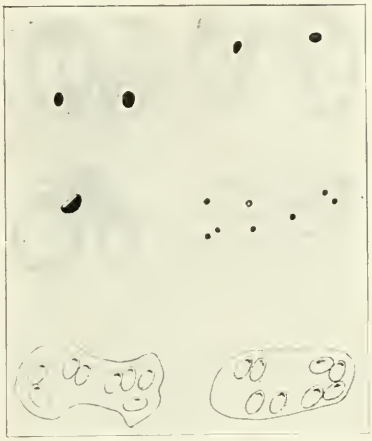


Fig. 2.—Zygosis and formation of an ascus with ascospores in Zygosaccharomyces octosporus (after Guilliermond).

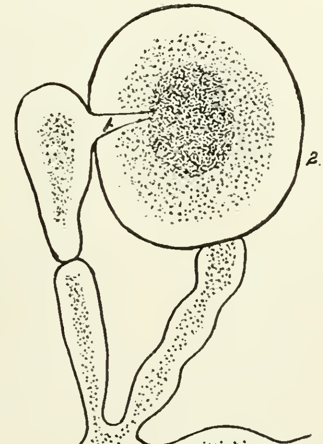


Fig. 3.—Sexual conjugation: (1) Antheridium; (2) Oösporangium.

Fungi are classified principally according to the manner in which the spores are produced, their shape and their color. There are several kinds of spores. The principal ones are the following:

1. *Gonidia* (endospores *sensu stricto*, ascospores *pro parte*).—These spores are not born free; they are formed usually by a highly complicated process, or born in a case or receptacle, or saclike cell, which is often terminal. When there are 2, 4, 8 or a multiple of 8 spores, they are generally called ascospores, and the receptacle is an ascus.

2. *Zygospores*.—These spores result from a conjugation of two cells (gametes) that are morphologically identical in shape, and do not show any sexual differentiation. The two gametes come into contact and unite, forming a large cell with a resistant double wall called "zygospore."

3. *Oösporcs*.—These result from the conjugation of two sexually differentiated elements or gametes: the male element, antheridium, and the female element, oösporangium or oögonium. The oösporangium generally has a thick capsule with pores, and contains several roundish protoplasmic masses: female gametes, macrogametes or oöspheres.

The male element (male gamete, antheridium) which originates on a special delicate hypha comes into contact with the oösporangium, sending a protoplasmatic process through it. The antheridium may divide into several motile bodies (antherozoids, spermatozoids) which fertilize the female gametes.

4. *Conidia* (or exospores).—These spores are born free, not in a saclike cell or case, or receptacle; the branch bearing them is called a conidiophore. Conidia originate asexually from the mycelial threads by a process of budding, septation or abstriction. Conidia may be pedunculated or nonpedunculated, lateral or terminal.

According to their size, conidia may be divided into macroconidia and microconidia. Among the macroconidia may be mentioned the so-called "fuseaux" or spindles of French authors, which are large conidia of a particularly differentiated type, being large, spindle-shaped, thick walled and sometimes septate transversely. The shape of these macroconidia is characteristic in the genus *Trichophyton* and the genus *Microsporum*.

Many authorities make a distinction between true conidia and pseudoconidia or aleuriospores. True conidia are easily detached from the mycelial hyphae which bear them, and when they have become free they originate new spores by a process of budding, or give rise to mycelial filaments by germination. True conidia are incapable of forming new spores or new mycelial threads while they remain attached to the parent mycelium. The mycelial hypha which carries the conidia is termed conidiophore or sporophore; a phialide is a flask-shaped segment

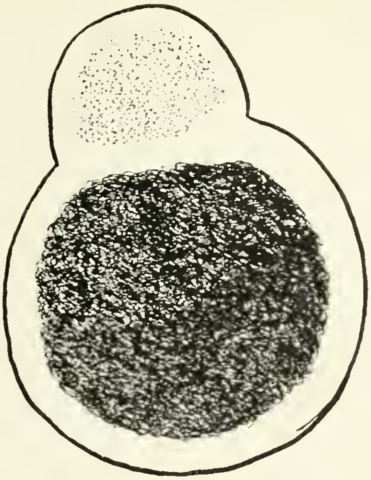


Fig. 4.—Blastospore.

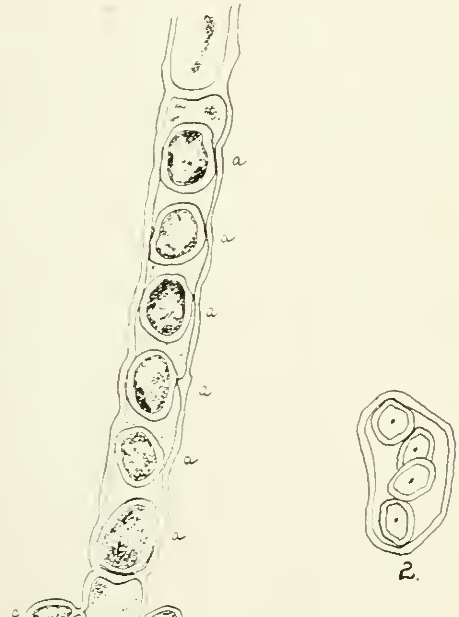


Fig. 5.—Endomyces vuillemini Landrieu, from a culture (after Vuillemin): (1) mycelial thread with arthrospores (*a*) and conidia (*c*): (2) ascus fructifications.

interposed between the sporophore and the conidia; a prophialide is a special article on the sporophore which supports several phialides.

The false conidia, or pseudoconidia, or aleuriospores, which may be lateral, terminal or intercalary, are not originally distinct from the thallus and are not easily detached; they are only set free by the death of the mycelial hypha to which they are attached.

5. *Thallospores.*—A thallospore is merely a portion of the thallus, or vegetative body, that becomes secondarily adapted to the purposes of reproduction. There are several varieties of thallospores, the principal ones being:

The blastospore, which is oval or roundish, is formed by a process of budding.

The arthrospore, or mycelial spore, is formed simply by the segmentation and disarticulation of a hyphal element or mycelial thread: it is at first square, and later becomes roundish or oval.

The chlamydospore is merely an arthrospore of large size that is undergoing encystment.

6. *Hemispores.*—The hemispore starts by a differentiation from the thallus, the "protoconidium," but this remains where it is formed, while the hypha continues to grow; later the protoconidium forms secondary functional spores which are called "deuteroconidia"; these are the true reproductive spores.

Classification of Fungi

Fungi may be separated into two large divisions:

A. Vegetative body under form of a multinucleate, naked plasmodium—*Myxomycetes.*

B. Vegetative body usually filamentous—*Eumycetes.*

Fungi parasitic of man belong to *Eumycetes,* which may be subdivided as follows:

Class I: Reproduction by freeborn spores (conidia) that are never contained in a case or receptacle; septate mycelium—*Fungi imperfecti* or *Hyphomycetes.*

Class II: Reproduction by ascospores; mycelium septate when present—*Ascomycetes.*

Class III: Mycelium nonseptate in vegetative stage; reproduction by zygospores—*Phycomycetes.*

Class IV: Reproduction by basidiospores; septate mycelium—*Basidiomycetes.* A basidium is a clublike hypha from the claviform portion of which two or four short delicate filaments originate, each of which bears a single spore (basidiospore).

CLASS I. FUNGI IMPERFECTI FUCKEL, 1869

The class *Fungi imperfecti* Fuckel, 1869, is most important from a medical point of view; it may be subdivided into (*a*) *Deuteromycetes* Saccardo, 1886 (accessory fructifications present), and (*b*) *Hyphales* Vuillemin, 1910 (accessory fructifications absent). Only the latter group contains fungi parasitic of man. It may be classified as follows:

Hyphales Vuillemin *Hyphomycetes* Fries	Order I.	*Microsiphonales* Vuillemin, 1912. (Hyphae bacilliform, 1 micron or less in diameter.)
	Order II.	*Thallosporales* Vuillemin, 1910. (Reproduction by thallospores.)
	Order III.	*Hemisporales* Vuillemin, 1910. (Reproduction by hemispores.)
	Order IV.	*Conidiosporales* Vuillemin, 1910. (Reproduction by conidia).

ORDER I. MICROSIPHONALES VUILLEMIN, 1912

Definition.—*Hyphales* with the mycelium composed of fine bacillus-like hyphae, usually 1 micron or less in diameter; generally gram-positive when young, and without distinct nuclei. Parasitic on man, animals and plants, or saprophytic.

Remarks.—This is a most interesting order, as the organisms belonging to it are nearly always mistaken at first sight for bacilli: hence the description of bacilli as the cause of so many fungal diseases due to these organisms.

The order may be divided into families as follows:

A. *Nocardiaceae* Castellani and Chalmers, 1918.

Synonyms.—*Actinomycetes* Lachner-Sandoval, 1898; *Trichomycetes* Petrusky, 1903.

Definition.—*Microsiphonales* with a mycelium.

Type Genus.—*Nocardia* Toni and Trevisan, 1889.

B. *Mycobacteriaceae* Miehe, 1909.

Definition.—*Microsiphonales* without a mycelium.

Genus 1.—*Mycobacterium* Lehmann and Neumann, with the tubercle bacillus as a type.

Genus 2.—*Corynebacterium* Lehmann and Neumann, with the diphtheria bacillus as a type.

Most authorities place *Mycobacteriaceae* in *Schizomycetes* (bacteria).

Family *Nocardiaceae* Castellani and Chalmers, 1918.

Synonyms.—*Actinomycetes* Lachner-Sandoval, 1898; *Trichomycetes* Petrusky, 1903.

Definition.—*Microsiphonales* with a mycelium.

Type Genus.—*Nocardia* Toni and Trevisan, 1889.

Classification.—Until recently all the species of this order were considered to belong to one genus, i. e., *Nocardia* Toni and Trevisan, 1889, but Pinoy has made a subdivision, separating certain species into another genus, which he calls *Cohnistreptothrix* Pinoy, 1911. In doing this, he points out that the original discoverers of actinomycosis, namely, Harz and Bollinger in 1877, and Rivolta in 1878, thought that they were dealing with one organism, but when they attempted to make cultures, it became apparent that more than one organism was implicated. Thus, Bostrom isolated a parasite which grew well aerobically, produced a dry membrane on the surface of broth and was capable of growth at 20 C. on gelatin, but which grew better on potato at 37 C., and formed chains of arthrospores.

Wolf and Israel, on the other hand, obtained a parasite which grew only anaerobically and was not capable of growth at ordinary European air temperature. In broth it formed small granules or scales which fell to the bottom of the tube. These cultures often contained clublike forms, and the branching filaments broke up into bacillar or coccal-like forms. Inoculation of gelatin cultures into the peritoneal cavity of guinea-pigs produced actinomycosis. This form is commonly called *Nocardia israeli* (Kruse, 1896). Wright maintains that this organism is the true cause of actinomycosis and that *N. bovis* is merely a contamination, but this is not generally accepted.

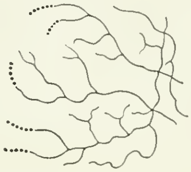


Fig. 6.—Nocardia buccalis (after Roger and Sartory).

There are, however, two distinct organisms which can cause actinomycosis in man and oxen, viz., *N. bovis* (Harz, 1877) and *N. israeli* (Kruse, 1896), but the difference between them is considerable, and therefore Pinoy has separated the latter and its allies from the former and has founded the new genus *Cohnistreptothrix* Pinoy, 1911. These two genera are distinguished as follows:

A. Grows aerobically, easy of cultivation, and produces arthrospores—genus 1, *Nocardia* Toni and Trevisan, 1889.

B. Grows best anaerobically, but can often grow aerobically, difficult of culture, and does not produce arthrospores—genus 2, *Cohnistreptothrix* Pinoy, 1911.

Classification of Genus Nocardia.—The numerous species of this genus may be separated into three sections:

I. Habitat, soil; can be found in air or water—section I, *Saprophytica* Foulerton, 1910.

Table 2.—*Diagnostic Classification of the Species of the Section Saprophytica, Subsection Majora*

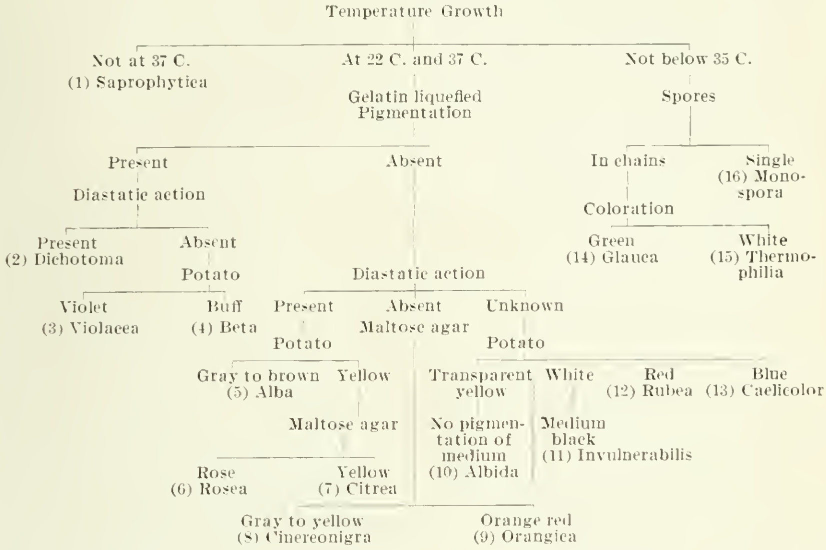
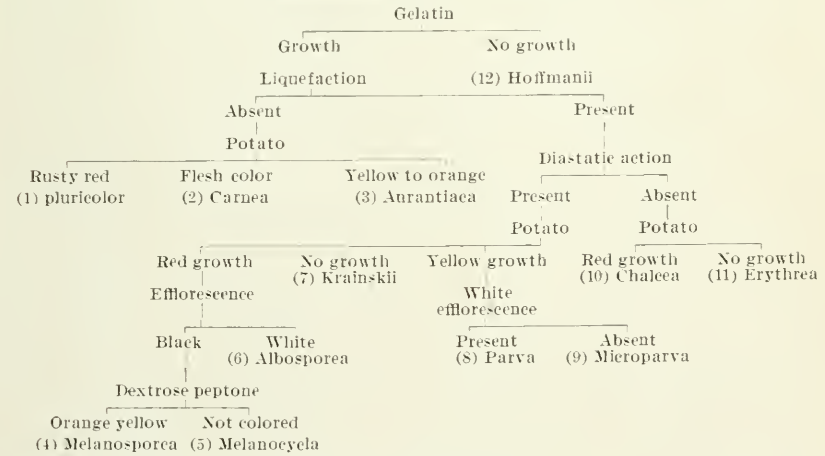


Table 3.—*Diagnostic Classification of the Species of Section Saprophytica, Subsection Minora*



II. Habitat, plants or animals—section II, *Parasitica* Foulerton, 1910.

III. Habitat, soil, plants or animals, but imperfectly described—section III, Incertae Sedis.

Section I, *Saprophytica* may be divided into two subsections:

(*a*) Majora, abundant growth at 22 C., well marked earthy or moldy smell, usually peptonize gelatin.

(*b*) Minora, growth less abundant, earthy or moldy smell absent or faint, gelatin rarely peptonized.

TABLE 4.—*Diagnostic Classification of the Species of the Section Parasitica, Subsection Majora*

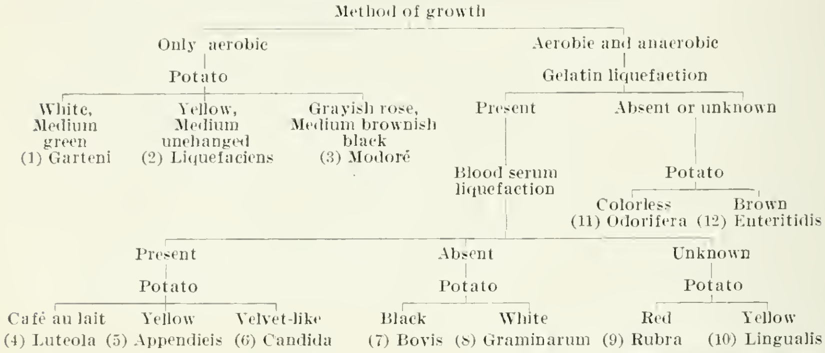
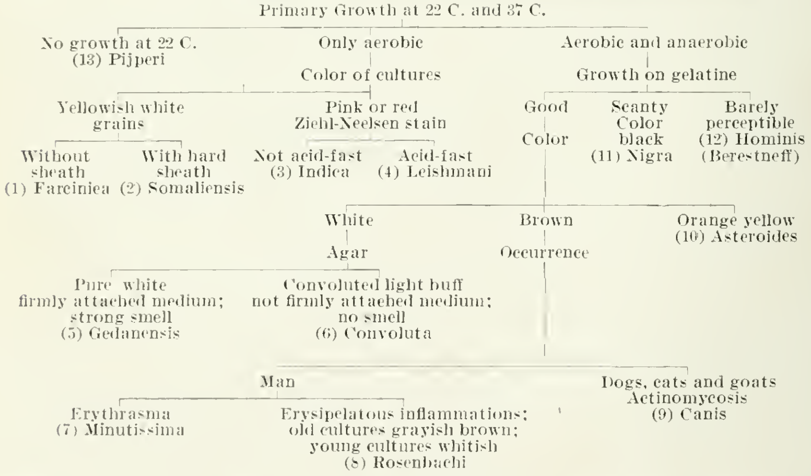


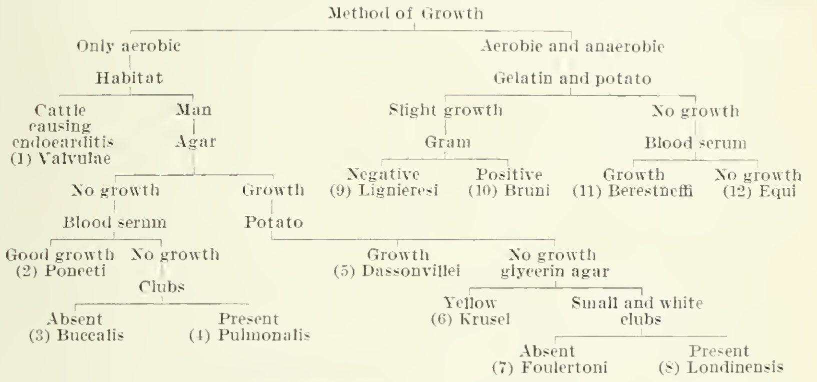
TABLE 5.—*Diagnostic Classification of the Species of Section Parasitica, Subsection Minora*



Section II, *Parasitica* may be separated into three subsections:

(*a*) Majora, growth at 22 C. spreading, efflorescence, bright, chalky.
(*b*) Minora, growth at 22 C. circumscribed, efflorescence, dull, powdery.
(*c*) Brevis, growth at 22 C. slight, efflorescence usually absent.

Table 6.—*Diagnostic Classification of the Species of the Section Parasitica, Subsection Brevis*



Genus *Cohnistreptothrix* Pinoy, 1911.

Definition.—*Nocardiaceae* growing best, or solely, anaerobically; usually difficult to cultivate; arthrospores absent.

Type species.—*Cohnistreptothrix israeli* (Kruse, 1896).

Classification.—The following are the principal species: *C. israeli* Kruse, 1896; *C. thibiergei* Pinoy and Ravaut, 1909; *C. foersteri* Cohn, 1874; *C. tenuis* Castellani, 1911.

1. *Cohnistreptothrix israeli* Kruse, 1896. Found in cases of human and bovine actinomycosis; differs from *Nocardia bovis* in being strictly anaerobic; pathogenic to laboratory animals producing fairly large yellow granules.

2. *Cohnistreptothrix thibiergei* Pinoy and Ravaut, 1909. Isolated by Pinoy and Ravaut from a peculiar nodular condition; may be cultivated aerobically and anaerobically; grains smaller than those produced by *C. israeli.*

3. *Cohnistreptothrix foersteri* Cohn, 1874. Found in concretions in the lachrymal canals; can be grown with great difficulty aerobically and anaerobically; colonies on maltose agar cerebriform, grayish.

4. *Cohnistreptothrix tenuis* Castellani, 1911. Produces a nodular condition of the hair of the axillary regions; in the nodules the organism appears in the shape of bacillary-like bodies, gram-positive, not acid-fast, packed together and embedded in an amorphous cementing substance; has not been cultivated.

A more complete description of the various species of *Nocardia* and *Cohnistreptothrix* may be found in Castellani and Chalmers' "Manual of Tropical Medicine," edition 3.[3]

A few words may be added on certain filamentous vegetal organisms, on the classification of which there is much discussion and which are hardly mentioned in textbooks, viz., organisms of the genus *Leptothrix*, of the genus *Cladothrix* and of the genus *Vibriothrix*.

Genus *Leptothrix* Kützing, 1843.

Definition.—Filamentous fungi with long, thin mycelial threads, and no capsule or only a delicate one; nonbranching, nonseptate, generally noncultivatable.

Type Species.—Leptothrix maxima, Miller.

The following species have been described:

Leptothrix maxima Miller, 1882. *Synonym.—L. buccalis maxima* Miller. Long thin filaments, unsegmented, or with long segments; when treated with iodine and dilute sulphuric acid gives a blue granulous reaction; has not been cultivated.

Leptothrix innominata Miller, 1882. Morphologically identical with *L. maxima,* but when treated with iodine and dilute sulphuric acid does not give a blue reaction; has not been cultivated.

Leptothrix racemosa Miller, 1882. Filaments somewhat thicker than those found in the two preceding species; on staining shows a peculiar beaded appearance; has not been cultivated.

Leptothrix placoides Dobrzyniecki. Long thin filaments, gram-positive, nonmotile; gelatin liquefied; growth on agar slow; produces hard granular colonies; isolated from human mouth by Dobrzyniecki.

Leptothrix filiformis Flexner, 1896. *Synonym.—Bacillus* (*Leptothrix?*) *pyogenes filiformis* Flexner, 1896. Isolated by Flexner from a rabbit; is nonmotile, of difficult cultivation, pathogenic.

Leptothrix vaginalis Donne, 1885. Found in vagina of women and animals.

Genus *Cladothrix* Cohn, 1875.

Definition.—Filamentous fungi with mycelial threads, long, thin, showing pseudobranching. The only well known species is *Cladothrix dichotoma* Cohn.

Cladothrix dichotoma Cohn, 1817. Long, thick mycelial threads straight or slightly undulating. They are not dichtomous, as the name would suggest; it is merely a case of pseudobranching. The organism can be cultivated on ordinary laboratory mediums, forming on agar a brownish, wrinkled, tough, membranous layer, very adherent. The medium may become stained, slightly brownish. The organism is found often in waters. I have found it, or a similar species, in an ulcer of the foot in association with many other organisms.

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[3] Castellani, Aldo, and Chalmers, A. J.: Manual of Tropical Medicine, ed. 3, New York, William Wood & Co., 1920.

Genus *Vibriothrix* Castellani, 1917.

The mycelial articles are of different shape: bacillary, vibrio-like, spirillum-like and at times club-ended. Globular or pear-shaped bodies of variable sizes may be present. The organisms belonging to this genus are generally motile; gram-negative, not acid-fast; cultivable on ordinary mediums; most of them aerobic (facultative anaerobic), some anaerobic.

Type Species.—Vibriothrix zeylanica Castellani, 1910.

Synonyms.—Spirillum zeylanicum Castellani, 1910; *Vibrio zeylanicus* Castellani, 1913; *Bacillus zeylanicus* Castellani, 1913; *Vibriothrix zeylanica* Castellani, 1917; *Spirobacillus zeylanicus* Castellani, Spagnolo and Russo, 1918.

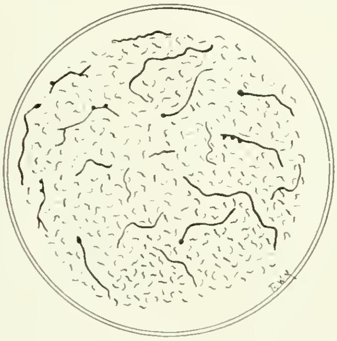

Fig. 7.—Vibriothrix zeylanica (preparation from water of condensation of dextrose agar culture).

Remarks—Polymorphic organism, vibrio-like bacillus-like and undulating forms being often found in the same preparation; small, medium-sized and occasionally large roundish bodies are at times observed, and clublike forms may also be present.

The organism is motile, gram-negative and not acid-fast. It is grown easily on ordinary mediums. On potato the growth is often reddish. In broth there is often a pellicle; preparations from fluid mediums generally show a predominance of vibrio-like or bacillary forms, while in the pellicle long undulating forms are often found. On MacConkey's medium the colonies are white, and somewhat resemble those of the typhoid-dysentery group. The organism does not ferment any of the usual laboratory carbohydrates or alcohols: dextrose, levulose, galactose, maltose, lactose, saccharose, mannitol, dulcitol and raffinose. Frequently, in fact, there is production of alkalinity. Milk is not clotted and is rendered alkaline, and certain strains after several weeks may induce a certain degree of peptonization. The great majority of strains are nonpathogenic to rabbits and guinea-pigs.

The germ was first isolated by me from cases of dysenteric enteritis in Ceylon, and has recently been observed by Spagnolo, Russo, Taylor, Douglas, Delamore and Alalon, Anigstein, Peruzzi and Ghiron, in Europe. Peruzzi and later Bhattacharzza have found that the organism has an ultramicroscopic phase.

The germ is found in great abundance in a number of cases of dysentery, while it seems to be rare in other conditions; it is doubtful, however, whether it can really become pathogenic, since I have found it also in cases in which the typical Shiga-Kruse bacillus was present. It may, perhaps, be considered a so-called "noso-parasite," as in the case of certain species of proteus found in cholera, typhus fever and other conditions.

An organism similar to *Vibriothrix zeylanica* has been found in cases of granular mycosis of the tonsils. Organisms somewhat similar to *V. zeylanica*, but some of them anaerobic, have been isolated from sputum by me and other observers.

ORDER II. THALLOSPORALES

The order *Thallosporales* Vuillemin, 1910—hyphales reproducing by means of thallospores—is divided into two suborders: *Blastosporineae,* in which reproduction takes place by means of blastospores, and *Arthrosporineae,* in which reproduction takes place by arthrospores.

The suborder *Arthrosporineae* would be important from a practical point of view, if the view of certain authorities were accepted, viz., that the *Trichophyton fungi* belong to it. I am inclined, however, to support the view that these fungi belong to *Ascomycetes* order *Aspergillales*, family *Gymnoascaceae* Baraketzky, 1872.

Suborder I, Blastosporineae Vuillemin, 1911

The suborder *Blastosporineae,* contains five principal families:

1. *Cryptococcaceae* Kützing.
2. *Oosporaceae* Saccardo.
3. *Enantiothamnaceae* Chalmers and Archibald.
4. *Haplographiaceae* Saccardo.
5. *Cladosporiaceae* Saccardo.

They may be recognized as follows:

A. Hyphae not manifestly different from the spores.
  I. Spores not in chains. Usually do not ferment carbohydrates with the production of gas—family 1, *Cryptococcaceae* Kützing, 1833.
  II. Spores in chains. Usually ferment carbohydrates with the production of gas—family 2, *Oosporaceae* Saccardo, 1886.

B. Hyphae manifestly different from the spores.

- I. Spores not in chains, but arranged verticillately—family 3, *Enantiothamnaceae* Chalmers and Archibald, 1915.
- II. Spores in chains—family 4, *Haplographiaceae* Saccardo, 1886.
- III. Spores in short chains or solitary—family 5, *Cladosporiaceae* Saccardo, 1886.

FAMILY 1: *Cryptococcaceae* Kützing, 1833.

Definition.—*Blastosporineae* in which the hyphae are little different from the conidia, both being yeastlike in form. The conidia are entirely formed by gemmation from the hyphae and are never in chains.

Classification.—The family contains the following genera: *Torula* Persoon, 1801; *Cryptococcus* Kützing, 1833; *Pityrosporum* Sabouraud, 1895; and *Mycoderma* Persoon, 1822, which may be differentiated as follows:

A. Vegetative elements not elongate.

- I. Nonpathogenic.
  - Vegetative elements roundish or oval, containing a large globule of fat; budding takes place, and often by several buds at the same time; pigment is present frequently; in fluid sugar mediums a thick pellicle, without bubbles of air, is slowly produced—*Torula*.
- II. Pathogenic.
  - (*a*) Vegetative elements with well-developed double contour; no large globule of fat; budding takes place with single bud at a time; no thick pellicle on fluid sugar mediums; cultivated —*Cryptococcus*.
  - (*b*) Vegetative elements often without double contour; not cultivated—*Pityrosporum*.

B. Vegetative elements elongate.

In fluid sugar mediums a thick pellicle containing bubbles of gas is quickly produced—*Mycoderma*.

Cryptococcus Kützing, 1833.

Definition.—*Cryptococcaceae* with vegetative elements not elongate; pathogenic, with well developed double contour, but no globule of fat and only one bud at a time; usually no formation of thick pellicle on fluid sugar mediums; can be cultivated.

Type Species.—*Cryptococcus hominis* Vuillemin, 1901.

Cryptococcus dermatitis Gilchrist and Stokes, 1898. *Synonyms.*—*Blastomyces dermatitis* Gilchrist and Stokes, 1898; *Cryptococcus gilchristi* Vuillemin; *Zymonema gilchristi* Beurmann and Gougerot.

The fungus in the affected tissues appears as large globular cells, from 10 to 16 microns in diameter with double contour, reproducing by budding. In cultures, which are usually white, besides these globular elements, rudimentary mycelial tubes may occasionally be found, presenting lateral or terminal conidia; asci are absent. The fungus does not ferment sugars, and a pellicle is not formed. Gelatin is not liquefied. There are probably several varieties of this fungus.

Cryptococcus hominis Vuillemin, 1901. *Synonyms.—Saccharomyces* (sp.?) Busse, 1894; *Atelosaccharomyces busse-buschki* of Beurmann and Gougerot, 1909; *Atelosaccharomyces rudeli* of Beurmann and Gougerot, 1911.

This organism was found in abscesses in a woman by Busse. In the pus the fungus presented itself in the shape of oval bodies, with a membrane which had a double contour. These elements were arranged in groups; each group was embedded in an amorphous substance and surrounded by a capsule. Culturally the fungus showed only roundish budding forms, no mycelium and no asci. Growth on solid mediums was generally white with at times a yellowish tinge. Gelatin was not liquefied. According to Sasagawa, this fungus produces acidity in glucose, levulose, mannibal and galactose. The organism was pathogenic to rabbits, white mice and dogs.

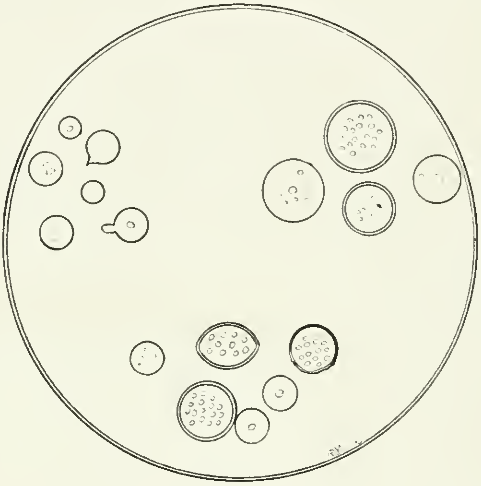

Fig. 8.—Cryptococcus hominis.

Cryptococcus epidermidis Castellani, 1914. Found by me in saccharomycosis or cryptococcosis epidermica; cells of variable size; has not yet been cultivated.

Cryptococcus farciminosus Rivolta and Miscellone, 1883. *Synonyms.—Cryptococcus tokishigei* Vuillemin; *Lymphosporidium equi* Gasperini, 1908; *Leukocytozoon piroplasmoides* Ducloux, 1908.

Definition.—Vegetative cells in situ, oval or roundish, with well marked double contour.

Remarks.—This organism is often included in hypertrophied endothelial cells and in leukocytes in the lesions in horses suffering from lymphangitis epizootica in Europe, Africa, Asia and America.

Cultivation.—It is difficult to cultivate. Nègre and Boquet have used with success a medium made of agar and dried horse dung. Sugar reactions are unknown.

Cryptococcus capsulatus Darling, 1906. *Synonym.*—*Histoplasma capsulatum* Darling, 1906.

Definition.—In the affected tissue the parasite appears in round or oval form, measuring from 1 to 4 microns in diameter and enclosed in an achromatic refractile capsule. Cultivation so far has been negative.

Remarks.—This organism was found by Darling in the endothelial cells of capillaries and small blood vessels in the lungs, spleen, liver, intestines and lymph glands, as well as in the leukocytes. It was considered by Darling and others to be a protozoon, and for it the genus *Histoplasma* was created. At present there is a consensus of opinion that it is a *Cryptococcus*.

Pathogenicity.—This fungus is pathogenic for man, causing disseminated, hyaline, pseudogranulomas in the lungs, splenomegaly, necrotic areas in the liver and ulceration of the small and large intestines.

Cryptococcus histolyticus Stoddard and Cutler, 1916. *Synonym.*—*Torula histolytica* Stoddard and Cutler. Found in a case of mycotic cerebrospinal meningitis (Freeman and Weidman); cultures yellowish. Produces acidity in glucose, maltose, saccharose and dextrine.

Cryptococcus ruber Demme, 1889. *Synonym.*—*Saccharomyces ruber* Demme, 1889.

This organism was found in the stools of children suffering from enteritis and also in certain specimens of milk.

It grows easily on ordinary laboratory mediums, producing red colonies. According to Casagrandi, it does not ferment any sugar.

Cryptococcus castellanii Re, 1925. *Synonym.*—*Monilia* (Sp.?) Castellani, 1924; *Monilia castellanii* Re, 1925. Isolated by me from peculiar cases of persistent furunculosis, not answering to staphylococcus vaccine.

Description (English cases).—Morphologically the organism is yeast-like, gram-positive and not acid-fast. The individual cells are somewhat rounded or oval. The maximum diameter of most cells is from 3 to 4 microns. The fungus is seldom detected microscopically in the pus of the boil-like lesions, staphylococci generally being present in large numbers; cultural methods are necessary; the cultures from the pus, as a rule, resemble cultures of *Staphylococcus aureus*. It may be necessary to make a dozen or more preparations from what looks like a pure staphylococcus growth to find a few yeastlike cells. By plating and replating on dextrose agar mediums the fungus is finally isolated.

Cultural and Biochemical Characters.—The fungus grows abundantly on dextrose agar and grows also well, though less abundantly, on ordinary agar. The cultures on dextrose agar are at first white, but after a few days they show a characteristic yellowish or yellowish brownish pigmentation, with occasionally a purplish or reddish tinge. Gelatin and serum are not liquefied.

Lead agar is not darkened during the first two weeks; later a slight darkening may occasionally take place.

Sugar Peptone Waters.—The fungus when recently isolated has little or no fermentative action on any sugar; it may produce a slight acidity in dextrose and levulose, and occasionally a slight amount of gas. When the fungus has been transplanted several times its fermentative action is greatly increased, and it generally produces acid and gas in dextrose and levulose and occasionally slight acidity in galactose; it does not form gas in any other sugars (table 1).

Litmus Milk.—The medium is not clotted; a slight acidity may be present.

Agglutination.—The fungus is agglutinated by the patient's blood in certain cases up to 1 in 400.

Ascospores and Mycelium.—I have never observed asci even in extremely old cultures; mycelium in small amount is not rarely present in certain mediums, especially fluid mediums. A more complete description may be found in the *Journal of Tropical Medicine and Hygiene*, June 15, 1925.

Cryptococcus metaniger Castellani, 1926. Isolated by me from a peculiar variety of *Trichomycosis nigra*. The cells are rather elongated, and it is possible that further investigation will show that the fungus does not belong to the genus *Cryptococcus*. On glucose agar it produces a jet black growth. It differs from *C. niger* Vuillemin in that the cells are more elongated and that the cultures are black in all the ordinary laboratory mediums. *C. niger* Vuillemin was originally found by Muffucci and Sirleo in 1895 from a pulmonary myxoma of a guinea-pig. The cells in situ have a thick mucilaginous membrane. The growth was brown or black on potato, but white on most other mediums.

Cryptococcus pararoseus Castellani, 1926. Isolated by me from the sputum of patients in the tropics with chronic bronchitis. Grows well on all ordinary mediums. The growth has a smooth surface and is pink or pink-red. It produces slight acidity in glucose levulose.

Cryptococcus rubrorugosus Castellani, 1926. Isolated from scrapings of the skin of the axillary regions. Grows abundantly on all the usual laboratory mediums; the growth has a crinkled surface and is red or bright pink-red.

Cryptococcus graciloides Castellani, 1925. Isolated by me from cases of stomatitis cryptococcobacillaris in Central America. It is generally found living in symbiosis with *Bacillus B. vermiculoides* Castellani, 1925, from which it may be separated with difficulty.

This cryptococcus is a delicate yeastlike organism, gram-positive and not acid-fast. Each cell is roundish or more often oval, with frequently a flasklike shape. In preparations from the cultures the average maximum longitudinal diameter of most cells is from 3 to 4.5 microns, and the maximum average transverse diameter is from 2 to 3 microns.

Cultural and Biochemical Characters.—On dextrose agar, minute colonies somewhat streptococcus-like appear. On McConkey lactose agar there are minute colonies, but growth is often more abundant than on dextrose agar. In gelatin and serum growth is exceedingly scanty, and neither of the mediums is liquefied. With lead agar there is no evident darkening of the medium.

Sugar Peptone Waters.—There is no fermentation of any sugar, but it must be noted that the fungus grows scantily in these mediums and that sometimes there is no growth.[4]

Cryptococcus macroglossiae Castellani, 1925. In Ceylon during the period 1903-1915 I observed three cases of a peculiar type of macroglossia of blastomycetic origin. Two years ago an interesting case of macroglossia of the same type was referred to me in London by Dr. Broughton Alcock, and in this case, too, I isolated a yeastlike organism. A full account of the case from a clinical point of view will be given at an early date by Dr. Alcock and me.

Description of the Organism.—It is a yeastlike fungus, gram-positive and not acid-fast. The individual cells are oval and of rather large dimensions, the maximum diameter varying between 4 and 5 microns.

Cultural and Biochemical Characters.—On dextrose agar there is abundant growth, white, with a smooth surface. On ordinary agar there are the same characters as on dextrose agar, but they are less abundant. Glycerin and serum are not liquefied.

Sugar Peptone Waters.—The organism does not produce gas in any sugar or other carbohydrate. Rarely it may produce a slight amount of acidity in dextrose.

Litmus Milk.—In litmus milk there is no change.

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[4] Castellani, Aldo: J. Trop. Med. **28**:217 (June 15) 1925.

Agglutination.—It is agglutinated by the patient's blood (1 in 400); not agglutinated, or only slightly so, by the blood of patients suffering from furunculosis blastomycetica.

Presence of Asci and Mycelium.—Asci have never been observed even in very old cultures; a little mycelium is not rarely visible in certain cultures, especially in fluid mediums. It would, therefore, probably be more correct to place this fungus in the genus *Monilia,* instead of the genus *Cryptococcus.* If it is placed in the genus *Monilia* it belongs to the zeylanica type (absence of gas in every sugar); it differs from *Monilia zeylanica* in not producing pigment, the cultures of *Monilia zeylanica* being yellow The correct name for the organism would be *Monilia macroglossiae* Castellani (synonym, *Cryptococcus macroglossiae* Castellani).

Remarks on Macroglossia Blastomycetica.—The whole tongue is greatly enlarged, occasionally painful; the patient feels discomfort in eating and may have some difficulty in speaking; he cannot whistle. It may be difficult to make the clinical diagnosis of blastomycetic macroglossia, as the tongue, though greatly enlarged, may show almost complete absence of the verrucoid patches so typical of blastomycosis. Deep scrapings should be made from the tongue after it is dried and painted with tincture of iodine, and cultures should be made. The observation of yeastlike organisms in superficial scrapings of the tongue is without importance, as these fungi are not rarely found in that situation even in normal mouths. Potassium iodide has a beneficial effect on the condition. Vaccines prepared with the fungus seem to be useful.

There are many other species of less known cryptococci. The following may be mentioned, none of which has been completely investigated biochemically.

Cryptococcus linguae-pilosae Lucet, 1901.
Cryptococcus plimmeri Costantin, 1901.
Cryptococcus degenerans Vuillemin, 1896.
Cryptococcus corsellii Neveu-Lemaire, 1908.
Cryptococcus hessleri Rettger, 1904.
Cryptococcus breweri Verdun, 1912.
Cryptococcus tonkini Legendre, 1911.
Cryptococcus sulfureus Beauverie and Lesieur, 1912.
Cryptococcus lesieuri Beauverie and Lesieur, 1912.
Cryptococcus salmoneus Sartory, 1911.
Cryptococcus niger Vuillemin.
Cryptococcus guillermondi Beauverie and Lesieur, 1912.
Cryptococcus harteri Beurmann and Gougerot, 1913.
Cryptococcus hudelo Beurmann and Gougerot, 1914.
Cryptococcus membranogenes Steinhouse, 1916.

Genus *Pityrosporum* Sabouraud, 1895.

Synonym.—Dermatophyton Dodd, 1910.

Definition.—Cryptococcaceae without well developed double contour.

Type Species.—Pityrosporum ovale Bizzozzero, 1882.

Remarks and Classification.—This genus, which is difficult to classify, is allied in appearance, in some forms, to a budding yeast, and as such comes close to *Cryptococcus.* So far, a species has not been cultivated, but two are known: viz., *P. ovale* Bizzozzero, 1882 (synonyms, *Saccharomyces capillitii* Oudemans and Pekelharing, 1885; *S. sphericus* Bizzozzero, 1884; *S. ovalis* Bizzozzero, 1889; *Pityrosporum malassezi* Sabouraud, 1895, often called the bottle bacillus of Malassez, in cases of pityriasis simplex capitis and pityriasis alba in Europe), and *P. cantlici* Castellani, 1908, in cases of seborrhea capitis in children in Ceylon.

They may be distinguished as follows:

A. Flask-shaped or oval, from 3 to 15 microns, but usually small—*Pityrosporum ovale.*

B. Generally roundish, from 5 to 16 microns, but usually large—*Pityrosporum cantlici.*

Family 2: *Oosporaceae* Saccardo, 1886.

Definition.—Blastosporineae in which the hyphae may be long or little different from the spores, which are typically in chains.

Classification.—This family is divided into several genera, the principal ones being *Oöspora* Wallroth, 1833, *Malbranchea* Saccardo, 1882, *Monilia* Persoon, 1797, and *Oidium* Link, 1809, which may be distinguished as follows:

A. Hyphae thin, short, simple or nearly simple.
  I. Terminating in chains of spores—*Oöspora.*
  II. Spores not in chains—*Malbranchea.*
B. Hyphae not thin, often long and branched.
  I. Sporophores simple or subsimple, typically with disjunction apparatus; dextrose completely fermented, gas being produced; numerous budding forms in cultures—*Monilia.*
  II. Sporophores simple, septate often with disjunction apparatus; dextrose not completely fermented, gas not being produced; budding forms rare or absent in cultures—*Oidium.*

These genera will now be briefly described.

Genus *Oöspora* Wallroth, 1833.

Definition.—Oösporaceae with a lax or compact mycelium in which the hyphae are slender, septate and marked by differentiated nuclei. The fertile hyphae are short, slender and nearly simple. The conidia, which are globose or ovoidal, hyaline or brightly colored, are arranged regularly in chains.

Remarks.—Saccardo in his "Sylloge Fungorum" recognizes a large number of species as belonging to this genus, and these are grouped into sections by the varying color of the conidia.

The only species known to cause disease in animals referable to this genus is *Oöspora canina* Sabrazes, 1893, which causes favus in dogs, and which can produce an eruption resembling ringworm when inoculated into man. According to Sabouraud, however, it has never been known to cause disease in man spontaneously (i. e., without experimental inoculation), and is, therefore, of little importance at present.

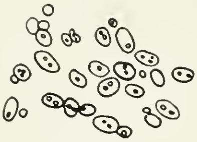

Figure 9

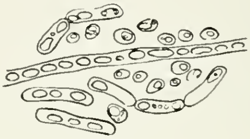

Figure 10

Fig. 9.—Monilia (preparation made from dextrose agar culture).
Fig. 10.—Monilia (preparation made direct from lesion).

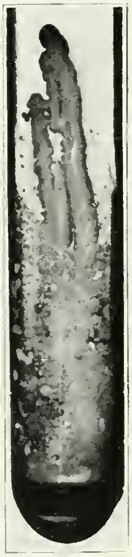

Fig. 11.—Monilia (culture on dextrose agar).

Vuillemin considers that *Achorion schoenleinii* Lebert, 1845, the fungus of favus of human origin, belongs to this genus.

The suggestion has been made that the fungi of tinea imbricata might also belong to this genus, rather than to the genus *Endodermophyton*.

Genus *Malbranchea* Saccardo, 1882. Spores are not in chains; arthrospores oval or cylindric and pinkish. The only species parasitic in man is *M. bolognesii-chiurcoi* Vuillemin, Pollacci and Nannizzi.

Genus *Monilia* Persoon, 1797.

Original Definition.—Stipitata and effusa byssoidea. Fila moniliformis articulata.

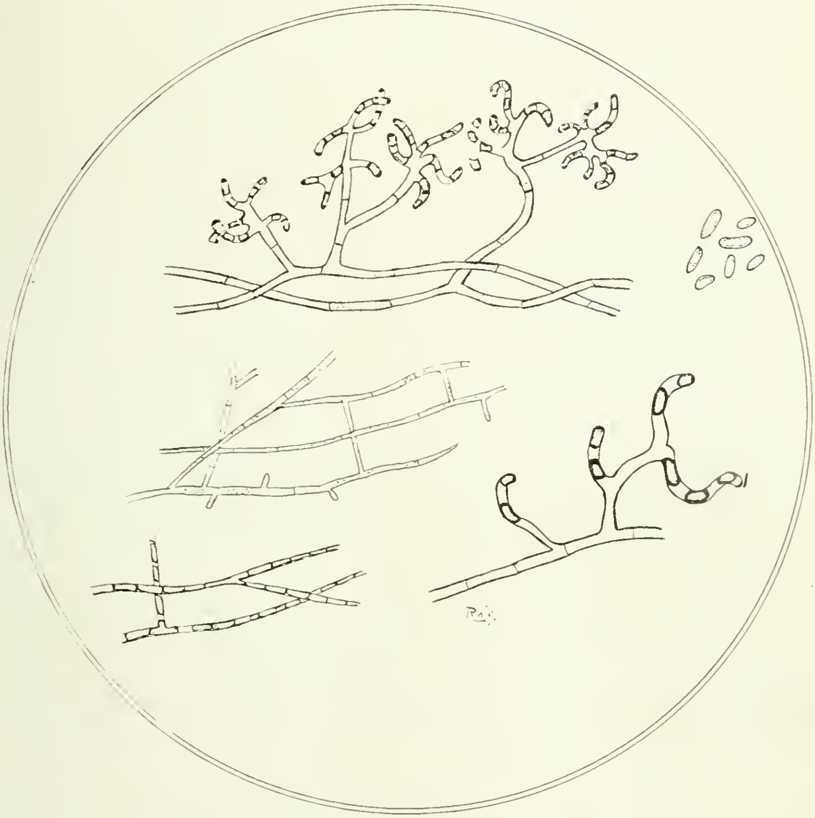

Fig. 12.—Malbranchea bolognesii—Chiurcoi, Vuillemin, Pollacci and Nannizzi (from Pollacci).

Botanic Definition.—Sporophores simple or subsimple, producing by constriction at their extremities a chain of large lemon-shaped conidia, often provided with a disjunction apparatus.

Usual Definition.—*Oösporaceae* possessing in situ budding forms and mycelial threads, which latter are often long and branched; in cultures mostly budding forms, but sometimes filaments, in which thallospores of the blastospore type are formed. Dextrose and often other carbohydrate mediums fermented with the production of gas.

Type Species.—It is difficult to know which is really the type species of this genus, but probably it is *Monilia aurea* Link, 1791.

Pathogenicity.—Fungi of this genus are the etiologic agents of thrush, some types of bronchomycoses and some dermatomycoses. It is practically impossible to differentiate the various species on morphologic grounds, and therefore, some years ago, I suggested a biochemical classification as follows:

1. Gas produced in dextrose only. *Balcanica Group.*—Principal species: *Monilia balcanica,* Castellani; *Monilia parabalcanica,* Castellani.
2. Gas produced in dextrose and levulose. *Krusei Group.*—Principal species: *Monilia krusei,* Castellani; *Monilia parakrusei; Monilia castellanii* Re. This is probably a cryptococcus.
3. Gas produced in dextrose, levulose and maltose. *Pinoyi Group.*—Principal species: *Monilia pinoyi* Castellani: *Monilia nabarroi* Castellani.
4. Gas produced in dextrose, levulose, maltose and galactose. *Metalondinensis Group.*—Principal species: *Monilia metalondinensis* Castellani; *Monilia pseudometalondinensis* Castellani.
5. Gas produced in dextrose, levulose, maltose, galactose and saccharose. *Tropicalis Group.*—Principal species: *Monilia tropicalis* Castellani; *Monilia metatropicalis* Castellani.
6. Gas produced in dextrose, levulose, galactose and saccharose. *Rhoi Group.*—Principal species: *Monilia rhoi* Castellani.
7. Gas produced in dextrose, levulose, maltose and saccharose. *Bronchialis Group.*—Principal species: *Monilia bronchialis.*
8. Gas produced in dextrose, levulose, saccharose and raffinose. *Guillermondi group.*—Principal species: *Monilia guillermondi* Castellani; *Monilia pseudoguillermondi* Castellani.
9. Gas produced in dextrose, levulose, galactose, saccharose and inulin. *Macedoniensis Group.*—Principal species: *Monilia macedoniensis* Castellani; *Monilia macedoniensoides* Castellani; *Monilia chalmersi* Castellani.
10. Gas produced in lactose and other carbohydrates. *Pseudotropicalis group.*—Principal species: *Monilia pseudotropicalis* Castellani, of which there are several varieties.
11. Gas produced in dextrin, in addition to other carbohydrates, but not in lactose. *Pseudolondinensis Group.*—Principal species: *Monilia pseudolondinensis* Castellani; *Monilia pseudolondinoides* Castellani; *Monilia africana* Macfie.
12. No gas produced in any sugar. *Zeylanica Group.*—Principal species: *Monilia zeylanica* Castellani; *Monilia zeylanoides* Castellani; *Monilia macroglossiae* Castellani.

It is important to note that many monilias after a few transplantations lose some of their fermentative characters or these are altered. Hence, the determination of species is possible only when recently isolated strains are used. There are, however, certain monilias as, for instance, *M. krusei, M. metalondinensis, M. tropicalis* and *M. macedoniensis,* which possess permanent biochemical characters.

TABLE 7.—*Biochemical Characters of Certain Monilias* *

	Dextrose	Levulose	Maltose	Galactose	Saccharose	Lactose	Mannitol	Dulcitol	Dextrin	Raffinose	Arabinose	Adonitol	Inulin	Sorbitol	Starch	Glycerin	Inositol	Salicin	Amygdalin	Isodulcitol	Erythrite	Gelatin	Serum	Litmus Milk	Color of Growth on Dextrose Agar
Monilia zeylanica Castellani	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	Yellowish
Monilia zeylanoides Castellani	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White†
Monilia (Cryptococcus) macroglossiae Castellani	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White‡
Monilia balcanica Castellani	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia parabalcanica Castellani	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia krusei Castellani	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia parakrusei Castellani	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia (Cryptococcus) castellanii Re	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Yellowish or reddish brownish
Monilia pinoyi Castellani	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia nabarroi Castellani	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia metalondinensis Castellani	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia pseudolondinensis Castellani	G	G	G	G	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia alba Castellani	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia pseudolondinoides Castellani	G	G	G	G	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia africana Macfie, 1921	G	G	G	0	G	0	0	0	G	G	0	—	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia albicans Robin, emendavit Castellani and Chalmers	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	White
Monilia tropicalis Castellani	G	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia metatropicalis Castellani	G	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia rhoi Castellani	G	G	0	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia guillermondi Castellani	G	G	0	0	G	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia macedoniensis Castellani	G	G	0	G	G	0	0	0	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	C	White
Monilia macedoniensoides Castellani	G	G	0	G	G	0	0	0	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	White
Monilia chalmersi Castellani	G	G	0	G	G	0	0	0	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	Dark brown
Monilia bronchialis Castellani	G	G	G	0	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	White
Monilia pseudotropicalis I Castellani	G	G	0	G	G	G	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	0	C	White
Monilia pseudotropicalis II	G	G	0	G	G	G	0	0	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	White
Monilia pseudotropicalis III	G	G	0	G	G	G	0	0	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	C	White
Monilia pseudotropicalis IV	G	G	0	G	G	G	0	0	0	G	0	0	G	0	0	0	0	0	0	0	0	0	0	0	White

* G indicates production of gas; C, production of clot; 0, negative result; - , gas absent, no clotting.

† Does not produce any discoloration of lead agar.

‡ Produces black discoloration of lead agar.

Further details on classification of the various species of the genus *Monilia* may be found in the Manual of Tropical Medicine.[5]

Pathogenicity and Virulence.—The subcutaneous, intravenous or intrapulmonary inoculation of some monilias into rabbits does not produce any symptoms; other monilias are virulent and cause the death of the rabbit.[6] It is interesting to note that monilias with identical biochemical reactions may vary enormously in their virulence: some strains may be quite avirulent, while others are exceedingly virulent.

Genus *Oidium* Link, 1809, emendavit Pinoy.

Definition.—*Oösporaceae* with hyphae terminating in chains of spores; hyphae long and branched; sporophores simple, septate, often without disjunction apparatus; do not produce gas in carbohydrates.

Type Species.—*Oidium lactis* Link, 1809.

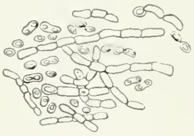

Figure 13

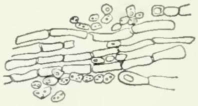

Figure 14

Fig. 13.—Oidium rotundatum (from a culture).
Fig. 14.—Oidium asteroides (from a dextrose agar culture).

Remarks.—Pinoy has adjusted this genus, so that now it contains human parasites, which are:

Oidium lactis Link, 1809.
Oidium rotundatum Castellani, 1911.
Oidium asteroides Castellani, 1914.
Oidium matalense Castellani, 1915.

These fungi may be differentiated as follows:

A. Produces only slight acidity, and only at times, in dextrose; cultures white —*Oidium lactis*.

B. Produce acidity in several carbohydrates.
  I. No acidity in dextrose; culture white and hairy—*Oidium matalense*.
  II. Acidity in dextrose.
    (*a*) No acidity in mannitol. Mycelial spores, roundish or oval; typical colonies convoluted—*Oidium rotundatum*.
    (*b*) Acidity in mannitol. Mycelial spores, quadrangular; typical colonies asteroid—*Oidium asteroides*.

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[5] Castellani and Chalmers (footnote 3, p. 1081).

[6] Castellani, Aldo: Fungi and Fungous Diseases: Lecture 2 (Bronchomycoses), Arch. Dermat. & Syph., to be published.

FAMILY 3: *Enantiothamnaceae* Chalmers and Archibald, 1915.

Definition.—Blastosporineae with a thallus composed of ramifying hyphae, with regular septums, which easily disappear, thus dissociating the segments, which are from 2 to 2.5 microns broad. The conidia are arranged verticillately around the septum.

Remarks.—This family contains only one genus, Enantiothamnus Pinoy, 1911.

Genus *Enantiothamnus* Pinoy, 1911.

Definition.—Enantiothamnaceae with the characters of the family.

Remarks.—There is only one species so far known, *Enantiothamnus braulti* Pinoy, 1911.

Enantiothamnus braulti Pinoy, 1911. *Definition.—Enantiothamnus* with oval conidia from 2 to 2.5 by from 1 to 1.5 microns.

Habitat.—Parasitic in man.

Remarks.—This fungus was discovered in an Arab in Algiers by Brault in umbilicated tumors containing pus, in the gluteal region.

On Sabouraud's agar the colonies are white, with a yellowish central portion.

Pathogenicity.—The organism produces tumors and pus in man, and is pathogenic for guinea-pigs.

FAMILY 4: *Haplographiaceae* Saccardo, 1896.

Definition.—Blastosporineae with hyphae manifest and distinct from the conidia, which are usually arranged in chains, or, in parasitic condition in man, in grapelike masses.

Remarks.—This family contains the general *Hormodendrum* Bonorden, 1851, and *Malassezia* Baillon, 1889, which are recognizable as follows:

All sterile hyphae creeping. Conidia all alike and produced on the hyphae. Conidiophores not spirally twisted; hyphae dendroid.

- A. Conidia in cultures in chains, in parasitic form in masses—genus *Hormodendrum* Bonorden, 1851.
- B. Conidia in parasitic form in masses; cultural form unknown—genus *Malassezia* Baillon, 1889.

Genus *Hormodendrum* Bonorden, 1851.

Definition.—Haplographiaceae with creeping dendroid sterile hyphae and branched conidiophores, bearing conidia all of one kind.

Remarks.—The genus *Hormodendrum* contains about ten species, which are generally parasitic on plants or saprophytic, but one is known to occur in man, viz., *H. fontoynonti.*

Type Species.—Hormodendrum olivaceum Corda, 1838.

Hormodendrum Fontoynonti Langeron, 1913. *Definition.—Hormodendrum* in cultures with sterile hyphae from 3 to 8 microns in diameter, greenish brown, septate with thick walls. The sporophores are well defined and not swollen at the extremity, and they carry chains of caducous spores. In man the mycelium is broken into long and undulating segments; spores, which are capable of budding, are arranged in masses.

Remarks.—This organism was found by Fontoynont and Carougeau in the scales of a dermatosis called "hodi-potsy" in Madagascar, which is comparable to that termed "tinea flava" in other tropical countries. It is doubtful whether the fungus is the etiologic agent of the condition.

Genus *Malassezia* Baillon, 1889.

Definition.—In man, mycelium broken into septate segments, with T-shaped or budding extremities. The hyphae carry round or oval conidia, which may be solitary or in grapelike masses, and may be smooth or with longitudinal, radial or spiral marks. Cultivation has not yet succeeded.

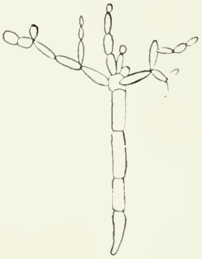

Fig. 15.—Hormodendrum.

Remarks.—This genus is known only to contain parasites of man. The two species may be recognized as follows:

A. Causing a brown eruption on the white skin, i. e., tinea versicolor; lesions superficial—species *Malassezia furfur* Charles Robin, 1853.

B. Causing a reddish yellow eruption on the white skin and a light yellow eruption on the dark skin, i. e., tinea flava; deeper lesions—species *Malassezia tropica* Castellani, 1905.

FAMILY 5: *Cladosporiaceae* Saccardo, 1886.

Definition.—Blastosporales with hyphae manifest and distinct from the conidia, which are either solitary or arranged in short chains.

Remarks.—Only the type genus *Cladosporium* Link need be considered; it may be recognized as follows:

Conidia smooth, not capitate, more or less in chains at first; hyphae and conidia uniform; hyphae not inflated, but decumbent; conidia in short chains and finally solitary—genus *Cladosporium* Link, 1809.

Genus *Cladosporium* Link, 1816.

Definition.—*Cladosporiaceae* with decumbent hyphae; conidiophores bearing smooth, uniform conidia arranged in short chains or solitary.

Remarks.—The type species is *Cladosporium herbarium* Persoon, 1801. A large number of species are scattered all over the world. They are commonly found on plants in tropical gardens, from whence the spores can easily be conveyed to the human skin, and either grow there, causing a lesion, or simply remain sheltered among the scales of other lesions, from which they may be grown in pure culture, thus giving rise to the impression that they may be causal organisms; however, they do not agglutinate with the patient's serum.

They may also grow as contaminations of laboratory mediums, so commonly may their spores be found in the air. The two species known in man may be recognized as follows:

A. Habitat, tinea nigra—species *Cladosporium mansoni* Castellani, 1908.
B. Habitat, ulcerating nodules—species *Cladosporium penicilloides* Gueguen, 1911.

SUBORDER II. ARTHROSPORINEAE VUILLEMIN, 1910

Definition.—Thallosporales with yeastlike forms, associated in cultures with hyphae and other forms with longer hyphae. Reproduction by means of arthrospores parasitic on man.

Remarks.—Vuillemin includes in this suborder the genera *Trichophyton, Microsporum, Achorion* and their allies, although it is more usual to consider these to be allied to the *Gymnoascaceae*, because *Ctenomyces serrata* Eidam, 1880, when injected into animals, develops a trichophyton-like mycelium and eruption, which classification has recently been supported by the work of Marshall and Chalmers on *T. currii*. However, for the present, it is proposed to leave these genera and their allies out of consideration.

Classification.—This suborder contains the following genera:

A. Producing piedra on hairs—genus *Trichosporum* Behrend, 1890.
B. Producing black maduromycosis—genus *Madurella* Brumpt, 1905.
C. Producing white maduromycosis—genus *Indiella* Brumpt, 1906.

Genus *Trichosporum* Behrend, 1890.

Definition.—Arthrosporales living parasitically on the hairs of man in the form of large, oval or roundish bodies embedded in a ground substance. In cultures elongated hyphae and spores are formed.

Remarks.—Care must be taken not to confuse *Trichosporum* Behrend, 1890, with *Trichosporium* Fries, 1849, a very different genus with over forty species, mostly saprophytic.

The species of this genus give rise to nodosities on hairs. They are:

T. giganteum Behrend, 1890, the cause of piedra in hairs of the head, in Colombia.

T. beigeli Rabenhorst, 1867, the cause of piedra in the hairs of the beard, in Europe.

T. ovoides Behrend, 1890, the cause of piedra, in hairs of the moustache.

T. ovale Unna, 1896, on the hairs of the moustache in Europe.

T. glycophile du Bois, 1910, on the pubic hairs of a diabetic patient.

The various species may be differentiated as follows:

A. Bodies around hair polyhedral.
    I. Diameter of bodies from 12 to 15 microns—*Giganteum.*
    II. Diameter of bodies from 3 to 4 microns—*Beigeli.*
B. Bodies around hair oval and small, from 3 to 4 by from 1.5 to 2.5 microns.
    I. In cultures hyphae often twisted like a corkscrew—*Ovale.*
    II. In cultures hyphae not so twisted—*Ovoides.*
C. Bodies around hair roundish, from 3 to 4 microns in diameter. Fungus associated with a coccus, with which it grows well on sugar mediums—*Glycophile.*

These fungi live as parasites on the surface of the hairs, but do not penetrate into their interior; during the parasitic life they vegetate in the shape of large oval or roundish elements, embedded in an amorphous substance. Saprophytically (cultures), they vegetate, forming mycelial threads and spores.

Genus *Madurella* Brumpt, 1905, emendavit Pinoy, 1912.

Definition.—Arthrosporineae with sterile septate hyphae, reproducing the thallus by fragmentation and secreting a black pigment. The spores are produced secondarily by binary division of the articles. They are found in black maduromycosis and grow well at 37 C.

Type Species.—Madurella mycetomi Laveran, 1902.

The principal species may be recognized as follows:

A. Sclerotia from 0.5 to 1 mm. in diameter formed in the depths of medium in cultures—*Mycetomi.*
B. Sclerotia rarely produced, and then on the surface of the medium—*Tozeuri.*
C. Numerous sclerotia on surface—*Americana* (Gammel).

Genus *Indiella* Brumpt, 1906.

Definition.—Arthrosporineae with septate, ramified hyphae, without black pigment, and hence sclerotia white or yellowish.

Type Species.—Indiella mansoni Brumpt, 1906.

Remarks.—White thallus, living as a parasite in various animal tissues—connective tissue, muscles and bones. Mycelial threads from 1 to 8 or 10 microns, septate, ramified, never secreting—in contrast to the fungi of genus *Madurella*—any black pigment. Masses of mycelial threads form sclerotia-like bodies or grains of various shape containing chlamydospores.

Classification.—The species may be recognized as follows:

A. Sclerotia hard and bean-shaped—*Mansoni.*
B. Sclerotia soft and in coiled masses.—*Reynieri.*

ORDER III. HEMISPORALES VUILLEMIN, 1910

Definition.—Hyphales with the mycelium composed of abundant hyphae, thin, but more than 1 micron in diameter, septate and branched conidiophores ramified basally, each branch terminating in a protoconidium, preceded by an annular construction produced by a brown, rigid thickening of the wall. The protoconidium is transformed completely or partially into deuteroconidia, but occasionally it elongates, forming a new conidiophore, or puts out branches which are capable of becoming conidiophores.

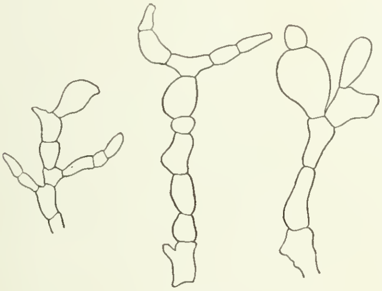

Figure 16

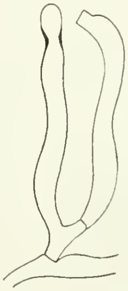

Figure 17

Fig. 16.—Madurella mycetomi (after Brumpt).
Fig. 17.—Hemispora stellata (after Vuillemin).

Classification.—There is only one genus, *Hemispora* Vuillemin, 1906.

Genus *Hemispora* Vuillemin, 1906.

Definition.—Mycelial filaments, thin, hyaline, septated, ramified. Some mycelial filaments show an ampulliform structure (protoconidium), which later divides into several sporelike segments (deuteroconidia).

The genus contains two species: asteroid colonies—asteroides, cerebriform—rugosa.

ORDER IV. CONIDIOSPORALES VUILLEMIN, 1910

Definition.—Hyphales with the mycelium composed of abundant hyphae, more than 1 micron in diameter, septate and branched, with or without true conidiophores, and with reproduction by means of true conidia.

Remarks.—The reasons for the formation of this order, which contains a large number of families, have already been given, as well as its advantage over the more fully worked out system of Saccardo.[7]

Classification.—The order *Conidiosporales* is divided into five suborders, which may be recognized as follows:

A. *Conidium imperfect:* Aleuriospore.
  Suborder 1: *Aleuriosporineae* Vuillemin, 1911.
B. *Conidium perfect:* Conidium verum.
  I. True conidiophores absent:
    Suborder 2: *Sporotrichineae* Vuillemin, 1910.
  II. True conidiophores present:
    (*a*) Conidia borne on sporophores.
    Suborder 3: *Sporophoralineae* Vuillemin, 1910.
    (*b*) Conidia borne on phialides.
      1. Prophialides absent.
    Suborder 4: *Phialidineae* Vuillemin, 1910.
      2. Prophialides present.
    Suborder 5: *Prophialidineae* Vuillemin, 1910.

The fourth suborder, *Phialidineae,* contains species of the genera *Aspergillus* Micheli, 1729, *Penicillium* Link, 1809, and *Sterigmatocystis* Cramer, 1859, in which asci are unknown; but though logically correct to include these species in the *Fungi imperfecti,* it is more convenient to consider them under the heading *Ascomycetes,* though the genus *Scopulariopsis* may be placed here.

The last suborder, *Prophialidales,* does not contain any genera with species parasitic in man.

Suborder I. Aleuriosporineae Vuillemin, 1911

Definition.—Conidiosporales in which reproduction takes place by aleuriospores.

Classification.—The suborder contains two families, which can be distinguished as follows:

A. Conidiophores absent—*Aleurismaceae.*
B. Conidiophores present—*Monotosporaceae.*

Family *Aleurismaceae* Vuillemin, 1911.

Definition.—*Aleuriosporineae* without conidiophores.

Classification.—The family is divided into two tribes:

A. Spores simple or appendiculate—*Aleurismeae.*
B. Spores bicellular or multicellular—*Blastotricheae.*

Only the first tribe is of interest to us.

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[7] Castellani and Chalmers (footnote 3, p. 1037).

Tribe Aleurismeae.

This tribe may be classified as follows:

A. Hyphae pale.
  - I. Hyphae short: sporogenous; apparatus but little differentiated from the mycelium—*Myceliophthora* Costantin, 1894.
  - II. Hyphae elongate, sporogenous apparatus but little differentiated from the mycelium—*Acladium* Link, 1809.
  - III. Hyphae elongate, a sporogenous apparatus well differentiated from the mycelium.
    - (*a*) Aleuriospore, smooth, small, acropleurogenous.
      - 1. Aleuriospores colored—*Aleurisma* Link, 1809.
      - 2. Aleuriospores pale—*Corethropsis* Corda, 1839.
    - (*b*) Aleuriospore, large, spiny, acrogenous.
      - 1. Aleuriospore, appendiculate *Mycogone* Link, 1809.
      - 2. Aleuriospore nonappendiculate—*Sepedonium* Link, 1809.

B. Hyphae dark.
  - I. Hyphae pale and dark; aleuriospores become dark and acropleurogenous on light or dark hyphae, small, generally 6 by 4, rarely 11 by 5, microns—*Glenospora* Berkeley and Curtis, 1876.
  - II. Hyphae dark; aleuriospores remain hyaline, situated acrogenously on hyaline hyphae at the base of sterile dark hyphae, large, from 11 to 14 microns—*Botryotrichum* Saccardo and Marchal, 1885.

Genus *Acladium* Link, 1809.

Definition.—*Aleurismeae* with pale elongate hyphae and with sporogenous apparatus, but little differentiated from the mycelium; sporophores unbranched; conidia pleurogenous.

Type Species.—*Acladium conspersum* Link, 1809.

Remarks.—There is only one species parasitic in man; viz., *A. castellanii.*

Acladium castellanii Pinoy, 1916. *Definition.*—*Acladium* with small chains of acrogenously placed chlamydospores. The aleuriospores are acropleurogenous.

Remarks.—The parasite was found by me in cases of ulcerarative dermatitis, with gumma-like nodules, in Ceylon, the Federated Malay States and Macedonia, and it was fully described by Pinoy.

The growth on artificial mediums (such as carrot, potato and dextrose agar) consists of many small roundish masses, which later on may coalesce. They are covered by spiculated formations, which give them a prickly appearance, and consist of erect, straight filaments, parallel to each other, or at times interlacing. These filaments are approximately 2 microns in diameter, and carry laterally pseudoconidia of variable shape, cylindriform, pyriform or spherical, attenuated in size at their points of insertion. Most of these pseudoconidia are 4 microns in length, with a breadth of 3 microns. This type of fructification recalls the type *Acladium* described by Bodin in certain species of the genus *Trichophyton* Malmsten, 1848.

These pseudoconidia become detached and then develop by sprouting, and mycelial filaments are formed. Certain filaments produce spherical chlamydospores arranged in small strings, as found in certain fungi of the genus *Fusarium*. These small chains of chlamydospores are frequently terminal, the dimensions being variable from 8 to 10 microns.[8]

In cultures on carrot and potato the colonies are white, on dextrose agar often amber. Old cultures may show a certain amount of pigmentation, brown or black, especially on potato.

Genus *Glenospora* Berkeley and Curtis, 1876.

Definition.—*Aleurismeae* with pale and dark hyphae. Aleuriospores become dark, and are situated acropleurogenously on light or dark hyphae, but are small, being generally 6 by 4 microns in diameter and rarely 11 by 5 microns.

Type Species.—*Glenospora curtisii* Berkeley and Desmond.

Classification.—Besides the type, *G. ramorum* Schweinitz, 1822, several pathologic species are known. The latter are:

G. graphii (Siebenmann, 1889, found in cases of otomycosis and keratomycosis).

G. sacchari Spegazzini, 1896, in the Argentine.

G. microspora Spegazzini, 1891, in Brazil.

G. khartoumensis Chalmers and Archibald, 1916, found in black maduromycosis.

G. semoni Chalmers and Archibald, 1917.

The various species may be recognized as follows:

A. Aleuriospores large, usually measuring 10 or more microns in diameter: Parasitic on plants—(1) *Curtisii,* (2) *Ramorum.*

B. Aleuriospores medium, measuring 6, 8, 9 or 11 microns; (3) *Sacchari.* Parasitic on plants—(4) *Microspora.*

C. Aleuriospores small, usually measuring 5 or less microns in diameter.
    I. Parasitic in man, causing otomycosis and keratomycosis—(5) *Graphii.*
    II. Parasitic in man, causing black maduromycosis.
        (*a*) Growth on clear maltose agar twelve days, uncapped tube at 30 C.; central black rounded mass surrounded by an abundant white fringe—(6) *Semoni* (in India).
        (*b*) Growth in exactly same conditions as in (*a*); central series of small elevations from which radiate furrows cutting in black plateau; fringe slightly marked—(7) *Khartoumensis* (in Africa).

Genus *Trichothecium* Link, 1824.

Fertile hyphae are erect, grouped together, each terminating in an oval, pear-shaped or globular conidium. The only species so far observed in man is *Trichothecium roseum.*

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[8] Castellani and Chalmers (fotnote 3, figs. 595-597).

Type.—Trichothecium roseum Persoon, 1801, emendavit Link, 1824.

Trichothecium roseum Persoon, 1801. *Synonyms.—Trichoderma roseum* Persoon, 1801; *Sporocephalum roseum* Persoon, 1801; *Puccinia rosea* Corda, 1837.

This species vegetates on decaying vegetable matter; the color is at first white, then pinkish. Each fertile hypha terminates in a pear-shaped conidium, from 10 to 20 microns in length and from 8 to 12 microns in breadth.

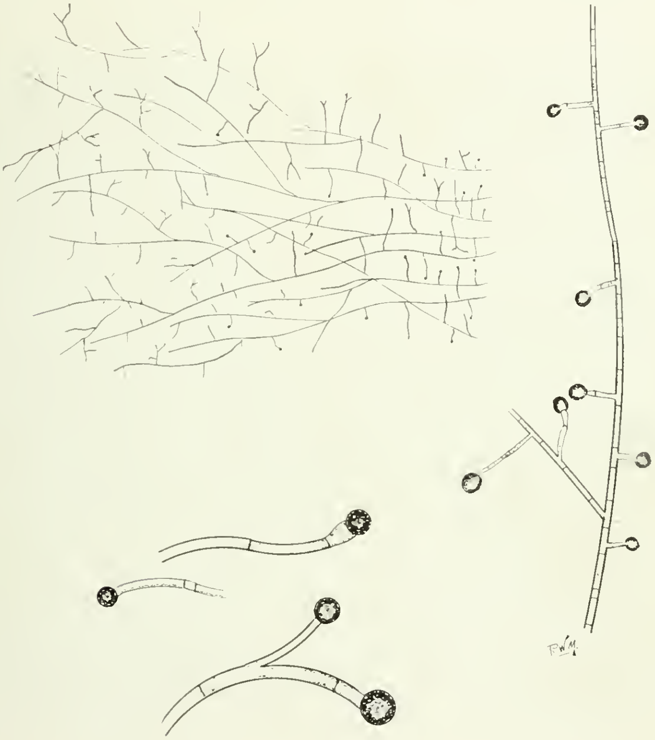

Fig. 18.—Acremoniella perinii Pollacci (from Pollacci).

This fungus has been found in cases of otomycosis by Stendener, who did not succeed in cultivating it. Some authorities believe it to be identical with *Glenospora graphii.*

Genus *Acremoniella*

In cultures, abundant, septate mycelium; each conidiophore bears a terminal dark brown spore from 7.7 to 9.7 microns in diameter.

SUBORDER II. SPOROTRICHINEAE VUILLEMIN, 1910

Definition.—Conidiosporales in which reproduction takes place by means of true conidia, but in which true conidiophores are absent.

Type Genus.—*Sporotrichum* Link, 1809, which has a large number of species scattered over the world, of which 100 are described in Saccardo's "Sylloge Fungorum," volume IV.

Genus *Sporotrichum* Link, 1809, emendavit Saccardo, 1882.

Synonyms.—*Miainomyces* Corda; *Chromelsporium* Corda.

Definition.—Sporotrichales with the characters of the suborder.

Human Species.—There has been considerable discussion as to whether the human species belonged to this or another genus, e. g., *Oöspora, Botrytis, Trichosporum, Rhinocladium* or *Torula*, but Smith, de Beurmann and Gougerot's belief that *Sporotrichum* is the correct genus has been supported by Matruchot, Vuillemin and Pinoy, and may be considered as settled as far as the present knowledge goes.

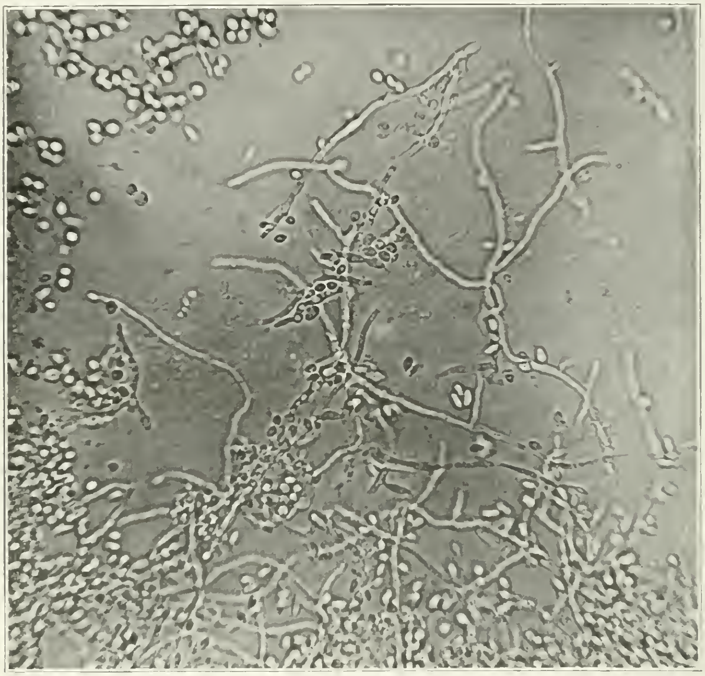

Fig. 19.—Sporotrichum schencki (courtesy of Dr. E. M. Johns).

The first species ever associated in any way with man was, *Sporotrichum inquinatum* Link, 1809, which was found growing on dry human feces. This was probably a saprophyte. The first ever found in diseased men was *Sporotrichum bronchiale* Montagne, 1844. It was discovered by Gubler in the bronchi of sick persons.

Species Parasitic in Man.—It is customary at present to give the following species as parasitic in man, and it may be noted that they do not correspond to any known species living saprophytically or parasitically on plants:

1. *S. schencki* Hektoen and Perkins, 1900.
2. *S. beurmanni* Matruchot and Ramond, 1905.
3. *S. dori* de Beurmann and Gougerot, 1906.
4. *S. indicum* Castellani, 1908.
5. *S. asteroides* Splendore, 1908.
6. *S. gougeroti* Matruchot, 1910.
7. *S. jeanselmi* Brumpt and Langeron, 1910.
8. *S. lesnei* Vuillemin, 1910.
9. *S. councilmani* Wolbach, Sisson and Meier, 1917.

And one may add:

10. *S. bronchiale* Montagne, 1844.

There is, however, some difference of opinion with regard to some of these species; thus, de Beurmann and Gougerot consider that the fourth and fifth are only varieties of the second, and many authorities (Davis, Hektoen, and others) consider that there are only two valid species: *S. schencki*, and *S. councilmani*, which may not even be a sporotrichum.

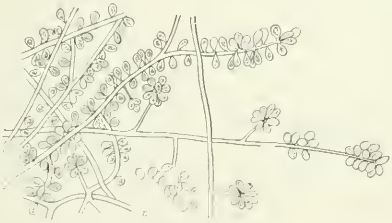

Fig. 20.—Sporotrichum (after Gougerot).

Considering them all provisionally as separate species, they may be recognized as follows:

A. Conidia large (from 4 to 11 microns in greatest diameter), with well developed pedicle and presence of lateral spore clusters:

Mycetomas in Madagascar—*Lesnei*.

Conidia large with absence of lateral spore clusters:

Traumatic arthritis in America—*Councilmani*.

B. Conidia usually small with short pedicle, which may be absent.

- I. With radiating bodies; conidia polymorphic, round, oval or bacilliform, varying from 4 to 8 microns in greatest diameter—*Asteroides*.
- II. Without radiating bodies.
  - (*a*) Conidia not numerous; ferments lactose, not saccharose—*Schencki*.
  - (*b*) Conidia numerous.

- 1. Hyphae 2 or more microns in diameter.
  - (A) Cultivated.
    - (1) Colonies dark from the first—*Gougeroti.*
    - (2) Colonies whitish at first.
      - (1^1) Colonies finally black; ferments saccharose, not lactose—*Beurmanni.*
      - (2^1) Colonies lightish brown—*Dori.*
      - (3^1) Colonies whitish gray to black; hyphae wide, from 3 to 4 microns—*Indicum.*
  - (B) Not cultivated; hyphae from 5 to 7 microns in diameter—*Bronchiale.*
- 2. Hyphae less than 2 microns in diameter; hyphae from 0.5 to 1 micron in diameter—*Jeanselmi.*

Pathogenicity.—These species usually give rise to lesions in the skin and deeper tissues that resemble those of tertiary syphilis (gummas, etc.) or tuberculosis, but *S. lesnei* produces a disease like mycetoma.

SUBORDER III. SPOROPHORINEAE VUILLEMIN, 1910

Definition.—Conidiosporales reproducing by true conidia borne on conidiophores.

Remarks.—A number of families and a large number of genera are included in this suborder, but one is concerned with only four, which may be separated from one another as follows:

- A. Conidiophores unbranched.
  - I. Single hyaline or lightly colored terminal spore—*Acremonium* Link, 1809.
- B. Conidiophores branched.
  - I. Conidiophores erect—*Monosporium* Bonorden, 1851.
  - II. Conidiophores decumbent—*Scedosporium* Saccardo, 1911.

Monosporium is mentioned only because *Scedosporium apiospermum* used to be *Monosporium apiospermum.*

Genus *Acremonium* Link, 1809.

Definition.—*Sporophorineae* with creeping sterile hyphae but little branched, and carrying laterally simple unbranched conidiophores, broad in the middle and gradually reduced toward the distal extremity, terminating in a single hyaline or lightly colored spore.

Type Species.—*Acremonium alternatum* Link, 1809.

Remarks.—A small number of species are known, which for the most part live saprophytically on decaying wood, leaves, etc. Two species only are known in man.

Acremonium petroni Vuillemin, 1911. Found by Potron and Noisette in a case of subcutaneous gummas with fever, which had somewhat resembled typhoid before the gummas appeared; easily grown on Sabouraud's agar; colonies white, then pinkish and, later, orange yellow; serum is liquefied. In cultures the mycelial filaments are septated; numerous conidiophores of a peculiar elongated type, from 15 to 20 microns in length, are present; conidia ovoid, with a smooth surface, from 4 to 5 microns in length and from 2 to 2.2 microns in breadth; pinkish. This fungus is pathogenic to guinea-pigs.

Acremonium niveum Boucher, 1918. Similar to *A. potroni*, but colonies are generally white.

Genus *Scedosporium* Saccardo, 1911.

Definition.—Sporophorineae with unbranched decumbent conidiophores.

Type Species.—*Scedosporium apiospermum* Saccardo, 1911.

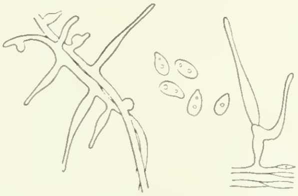

Fig. 21.—Acremonium petroni Vuillemin, 1911 (after Vuillemin).

Remarks.—The other known species are *Scedosporium acremonioides* Harvey, which is of no concern here, and *S. sclerotiale*.

Scedosporium apiospermum Saccardo, 1911. *Definition.*—*Scedosporium* with mycelium at first white and later slightly brown; bundles from 3 to 5 mm. broad in cultures; mycelial hyphae creeping, filiform; conidiophores decumbent, slightly branched, hyaline, from 2.5 to 3 microns, with one spore oblong, from 14 by 5.6 to 11 by 5.7, rarely subround; at first hyaline, later dilute, dirty rose yellow. *Scedosporium*, causing white maduromycosis.

Remarks.—Easily grown, the cultures are whitish and covered with duvet; may become brownish when old.

Mycelial tubes septated and of various size; may reach from 4 to 5 microns in breadth. Each conidiophore supports a terminal spore, which is either ovoid, from 12 to 14 microns in length, and from 5 to 6 microns in breadth, or roundish, from 6 to 7 microns in diameter. Other kinds of fructification are not seen.

Pathogenicity.—This fungus has been found in Italy, by Tarozzi and Radaeli, in cases of mycosis of the foot resembling madura foot.

Radaeli described it as a new species of the genus *Monosporium*. Saccardo placed it later in the genus *Scedosporium*. Radaeli made a complete pathologic and clinical investigation of the condition.

Scedosporium Sclerotiale Pepere, 1914. *Synonym.—Monosporium sclerotiale seu nigricans* Pepere, 1914.

Definition.—Scedosporium causing black maduromycosis.

Remarks.—This fungus resembles *S. apiospermum*, with the exception that it produces black pigment. It was ably investigated by Pepere, who grew it on various mediums, studied it mycologically, studied complement fixation with it and finally obtained successful inoculations in the anterior chamber of the eye in guinea-pigs.

Pathogenicity.—It was found in a case of black maduromycosis in a peasant, aged 33, who lived at Domusnovas, in the province of Cagliari, in Sardinia.

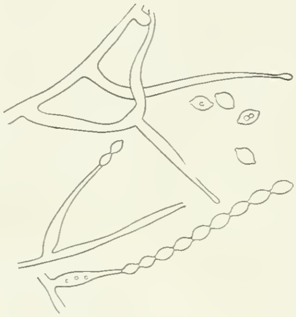

Fig. 22.—Scopulariopsis blochii Matruchot (after Vuillemin).

SUBORDER IV. PHILIDINEAE VUILLEMIN, 1910

Definition.—Conidiosporales with conidia borne on phialides.

Remarks.—As already stated, the species of *Aspergillus, Sterigmatocystis* and *Penicillium* in which asci are known could be classified here, but we will consider only *Scopulariopsis*, which is closely related to *Penicillium*.

Genus *Scopulariopsis* Bainier, 1907.

Synonym.—Penicillium pro parte.

Definition.—Phialidalineae resembling *Penicillium*, with unbranched erect conidiophores, bearing phialides, which carry a chain of large ovoid or roundish spores.

Type Species.—Scopulariopsis brevicaulis Saccardo, emendavit Bainier, 1907.

Remarks.—In addition to those found in man, *S. rubellus* Bainier, 1907, *S. rufulus* Bainier, 1907, *S. repens* Bainier and *S. communis* Bainier are known.

Species Found in Man.—Two species are found in man, which may be differentiated as follows:

A. With white creamy formation in old cultures—*Blochi.*
B. Without such formations in old cultures—*Komingii.*

(To be Continued)

FUNGI AND FUNGOUS DISEASES

LECTURE I *

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NEW ORLEANS

(Continued from page 425)

CLASS II. ASCOMYCETES

These fungi are characterized by their mode of reproduction, viz., by spores originating inside special cells called asci. The spores (ascospores, endospores, gonidia) inside the asci generally number four or eight or a multiple of eight. While these fungi live a parasitic life, asci are not found, and reproduction takes place by germination and conidia. The fungi belonging to this order are often pleomorphic, their morphologic characters changing according to the medium on which they live.

Classification.—Ascomycetes are divided into the following subclasses:

- A. Asci with varying number of spores, usually numerous—*Hemiascomycetes.*
- B. Asci with a definite number of spores.
  - I. Asci separate or scattered—*Protoascomycetes.*
  - II. Asci approximate, usually forming a hymenium—*Euascomycetes.*

SUBCLASS PROTOASCOMYCETES

The subclass *Protoascomycetes* contains a single order, the *Saccharomycetales.*

ORDER SACCHAROMYCETALES

This order is divided into two families as follows:

- A. Vegetative cells single or loosely attached in irregular colonies—*Saccharomycetaceae.*
- B. Vegetative cells forming a mycelium—*Endomycetaceae.*

Family *Saccharomycetaceae* Rees, 1870.

Definition.—Protoascomycetes with vegetative cells, single or loosely attached in irregular colonies; mycelium not usually developed; asci isolated, not differentiated from vegetative cells.

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* The Adolph Gehrmann Lectures of the University of Illinois College of Medicine, 1926.

Remarks.—These organisms are generally round or ovoid cells, presenting a cell wall of single or double contour; the internal protoplasmatic mass often shows granules and vacuoles. During active vegetation as parasites they reproduce by a budding process, hence the name of budding fungi; when living on artificial mediums, under unfavorable nutritive conditions, or in the absence of oxygen, they multiply also by formation of endospores or ascospores. Each cellular element may become an ascus containing from one to four, sometimes more, up to twelve spores (ascospores).

The organisms are, as a rule, unicellular plants, but at times the cells elongate, and a rudimentary mycelium may be formed. This occurs, for instance, when the organisms are cultivated in certain fluid culture mediums, for example, fluid beer-wort. In such cases sidebuds, which separate into conidia-like bodies, may also develop on the hyphae.

Classification.—The family contains thirteen genera, of which only three—*Saccharomyces* Meyen, 1837, *Debaryomyces* Klocher and *Willia* Hansen, 1904—contain species parasitic on man.

A^1. Vegetative cells globose, ovoid or pyriform, without lemon-shaped extremities.

B^1. Vegetative cells multiply by budding.

C^1. Vegetative cells without large fat globule; asci 3 to 4 spored.

D^1. Ascospores globose or ovoid.

E^1. On germination spores form typical yeast cells.

F^1. Ascus formation not preceded by zygosis.

G^1. Spore membrane single.

H^1. Spore membrane verrucose—*Debaryomyces* Klocher.

H^2. Spore membrane verrucose and possessing a superficial elevated linear process, dividing the surface into two unequal portions—*Schionniomyces* Klocher.

H^3. Spore membrane without verrucosities or crest—Saccharomyces *Meyen,* 1838.

G^2. Spore membrane double; an inner, endosporium, and an outer, exosporium —*Saccharomycopsis* Schionning.

F^2. Ascus formation preceded by zygosis; ascospores have a smooth membrane —*Zygosaccharomyces* Barker, 1901.

E^2. On germination, spores form a poorly developed promycelium, multiplication by a process intermediate between budding and transverse division—*Saccharomycodes* Hansen, 1904.

D^2. Ascospores pileiform or limoniform; costate with bowler hat shape; no alcoholic formation, but ethers produced, with pleasant fruity odor, in sugar mediums—*Willia* Hansen, 1904.

D^3. Ascospores spherical, hemispherical or irregularly shaped; thick pellicle with air-bubbles on sugar liquid mediums—*Pichia* Hansen, 1904.

C^2. Vegetative cells large, spherical, each containing a large fat globule as in torula; D. asci 8 spored—*Torulaspora* Linder.

B^2. Vegetative cells multiply by fission: asci 8 spored—*Schizosaccharomyces* Beyerinck, 1893.

A^2. Vegetative cells oval, with one or both extremities lemon-shaped; asci 1 spored—*Hansenia* Linder.

A^3. Vegetative cells elongate, cylindric, spores filiform; asci 1 spored—*Monospora* Metchnikoff; asci 8 spored; ascospores flagellate—*Nematospora* Peglion.

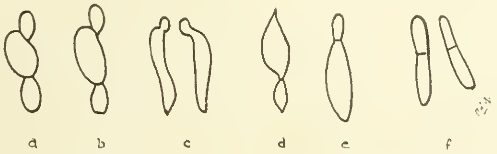

Fig. 23.—Types of yeast (vegetative cells): (*a*) Saccharomyces cerevisiae; (*b*) Saccharomyces ellipsoideus; (*c*) Saccharomyces pastorianus; (*d*) Saccharomyces apiculatus; (*e*) Saccharomycoides; (*f*) Schizosaccharomyces (after Lindner).

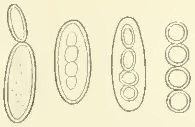

Fig. 24.—Saccharomyces anginae with ascospores (after Troisier and Achalme).

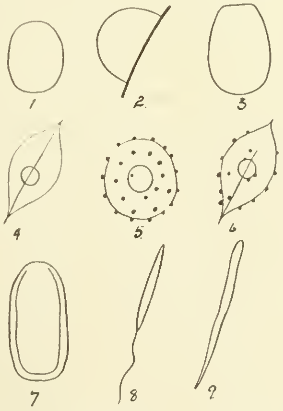

Fig. 25.—Various types of ascospores: (1) Saccharomyces; (2) to (4) Willia; (5) Debaryomyces; (6) Schiönnoimyces; (7) Saccharomycopsis; (8) Monospora; (9) Nematospora.

Genus *Saccharomyces* Meyen, 1838.

Definition.—Saccharomycetaceae with smooth spores, spore membrane single; no evidence of any copulative process; occasionally rudimentary mycelial tubes present, with transverse septation; induce alcoholic fermentation.

Remarks.—As already stated, the fungi of this genus, as well as of the genera *Cryptococcus, Monilia, Oidium* and *Coccidioides,* are usually called *Blastomycetes,* and the diseases induced by them blastomycoses.

The fungi of the genera *Saccharomyces, Cryptococcus* and *Coccidioides* have a great importance in dermatology, as they give rise to peculiar ulcerative conditions of the skin, often resembling a syphilide or a tuberculide. The first cases of blastomycosis were observed in America by Posadas, Wernike and others, who at first believed them to be a protozoal infection. Later, Gilchrist, Ricketts and others recognized the true nature of the parasites. Some authors (Sanfelice, Roncali, etc.) have considered cancer to be of blastomycetic origin.[9]

Saccharomyces blanchardi Guiart, 1906. Found at an operation by Blanchard, Schwartz and J. Binot, on a patient who had been considered to be suffering from tubercular peritonitis. The fungus had produced in the peritoneum a large, whitish, gelatiniform mass, weighing about 1 Kg. The fungus grew well on all sugar mediums.

On maltose agar it produced crateriform colonies of a snow-white color; asci with eight spores were present. On gelatin the growth was mucoid-like and gray. Liquefaction of the medium was slow. On potato there was a mucoid growth, whitish and darkening after a long time. On carrot the growth was viscid and abundant; it was pathogenic to rabbits, in which it induced a general mycosis, which terminated fatally.

Saccharomyces granulatus Vuillemin and Legrain, 1900. Observed by Vuillemin and Legrain in a tumor of the submaxillary bone; cells ovoid, from 4 to 5 microns in length, and from 3 to 4 microns in breadth; cultures pinkish or pinkish red; ascospores and chlamydospores present; gelatin not liquefied; sugar reactions not given.

Saccharomyces tumefaciens Curtis, 1896. *Synonym.*—*Saccharomyces subcutaneus tumefaciens* Curtis, 1896. Found by Curtis in a myxomatous tumor. It appeared in the tissues in the shape of spherical bodies from 16 to 20 microns in diameter, each surrounded by a zone of amorphous substance; grows well on all sugar mediums; in old cultures asci are seen, with from one to four spores; gelatin with surface growth whitish, no liquefaction; said to ferment saccharose, but not maltose or lactose; action on dextrose not mentioned; pathogenic to rats and dogs.

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[9] Fungi of the genus *Saccharomyces* are important from an industrial and agricultural standpoint, being the cause of alcoholic fermentation. The best known yeast is the brewer's yeast, *S. cerevisiae,* which is slightly ovoid, from 8 to 9 microns in diameter.

Saccharomyces samboni Castellani, 1907. Cells roundish, from 6 to 8 microns in diameter, easily grown on various mediums, producing white colonies, which rapidly coalesce; found by me in Ceylon in a few cases of intertriginous dermatitis of the cruroscrotal region. A similar or identical organism has been observed by Whitfield in England in a case of the same dermatitis. Ferments glucose, levulose and maltose; gelatin is not liquefied; in old cultures asci are occasionally present, but certain strains do not seem capable of producing asci and therefore probably belong to the genus *Cryptococcus.*

Saccharomyces hominis Klein and Gordon, 1903. Isolated in some cases of tonsillitis clinically resembling diphtheria; roundish or oval cells, from 5 to 7 microns in diameter; pathogenic to guinea-pigs and rabbits; sugar reactions unknown.

Saccharomyces anginae Achalme and Troisier, 1895. Found by Achalme and Troisier in a case of tonsillitis showing white patches; in situ cells ovoid, from 8 to 15 by 5 to 6 microns; in cultures 4 spored asci are present; on gelatin, surface growth white; ferments saccharose, but other reactions are not given.

Saccharomyces balzeri Balzer, Burnier and Gougerot, 1911. *Synonym.—Parendomyces balzeri* Balzer, Burnier and Gougerot, 1911. Grows on culture mediums under the type of oval or roundish, yeastlike cells, proliferating by budding; isolated by Balzer, Burnier and Gougerot from a gummatous condition; sugar reactions unknown.

Saccharomyces roseus Maggiora and Gradenigo, 1890. Found by Maggiora and Gradenigo in the eustachian tube, and considered by them to be nonpathogenic; cultures pink; sugar reactions unknown.

Saccharomyces cerevisiae Meyen, emendavit Hansen. *Synonym.—Cryptococcus fermentum* Kützing; *Torula cerevisiae* Turpin; *Hormiscum cerevisiae* Bail.

Cells oval or roundish, ascopores formed at all temperatures between 12 and 37 C., optimum from 25 to 30 C.; abundant growth on all ordinary laboratory mediums, colonies white, surface usually smooth. There are several varieties of *S. cerevisiae.* The one in my collection gas ferments dextrose, levulose, maltose, galactose and saccharose. This fungus and its varieties are used in the production of beer.

Saccharomyces ellipsoideus Hansen. Vegetative cells, mostly elliptical; optimum temperature for appearance of ascospores 25 C., but ascospore formation takes place at any temperature between 10 and 32 C. The ascus cell is of small dimensions, is ellipsoidal and contains from one to four ascospores which measure from 2 to 5 microns; according to Marchand, they germinate after having copulated two by two. This *Saccharomyces* which is found on the surface of grapes plays an important rôle in the process of vinification.

Saccharomyces pastorianus Hansen. Vegetative cells roundish or oval, at times elongated; asci elongated, usually containing from 1 to 4 ascospores; each ascospore varies in size between 1.5 and 3.5 microns; occasionally much larger ascospores may be seen, up to 5 microns in diameter; optimum temperature for development of ascospores 27.5 C., but ascospore formation takes place at any temperature between 4 and 30.5 C.; at 31.5 C. formation of ascospores ceases completely. This fungus gives a bitter taste and a bad odor to beer.

Genus *Debaryomyces* Klocher

Definition.—Saccharomycetaceae with ascospores presenting a verrucose surface. Several yeastlike organisms, some of which are found in scrapings from the skin and are probably nonpathogenic, have been classified in this genus by Ota. A pathogenic one is *Debaryomyces hudeloi* (de Beurmann and Gougerot, 1909), Fonseca emendavit 1922. Asci, which are scanty, contain one spore with a verrucose surface. The fungus is said to ferment glucose and saccharose, but not levulose, maltose, galactose or lactose. It was found by Hudelo, Rubens, Duval and Laederich in a case of multiple abscess in France (Busse-Buschke type of blastomycosis). The vegetative cells are roundish or oval, occasionally elongated. They are from 2 to 4 microns in diameter, and when elongated from 6 to 8 microns.

Genus *Willia* Hansen, 1904.

Definition.—Saccharomycetaceae with ascospores lemon-shaped or hat-shaped;[10] as a rule they do not produce alcoholic fermentation, but do produce various ethers; cultures present a pleasant fruity odor.

Species in Man.—Willia anomala Hansen, 1904

Willia anomala Hansen, 1904. Rather small oval cells; in sugar liquid mediums it forms a well marked membrane, containing air-bubbles; asci with from two to four spores of the so-called hatlike type; dextrose fermented, but not saccharose or maltose; ferments beer-wort, with production of ethers; found by Beauverie and Lesieur in the sputum of a tuberculous patient.

Family Endomycetaceae.

Definition.—Mycelium usually well developed, multiseptate; asci 4 to 8 spored; spores 1 celled.

A. Mycelium poorly developed—*Podocapsa*.

B. Mycelium well developed.

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[10] Castellani and Chalmers (footnote 3, fig. 492).

I. Asci formed after zygosis—*Eremascus.*
II. Asci formed asexually.
  (*a*) Asci 4 spored—*Endomyces.*
  (*b*) Asci 8 spored—*Oleina.*
  (*c*) Asci with a large number of spores—*Coccidioides.*

Only *Endomyces* and *Coccidioides* contain species parasitic of man.

Genus *Endomyces* Rees, 1870.

Definition.—*Endomycetaceae* with mycelium abundant, ramified or not, simple or septate; presence of budding and asci with four spores; reproduction by external spores, ascospores and spores situated inside the mycelial tubes.

Remarks.—Vuillemin believed at one time that the thrush fungus belonged to this genus. Researches carried out by me have demonstrated that the condition known as thrush may be caused by various fungi. These fungi, as a rule, do not reproduce by ascospores, and, as justly remarked by Pinoy and Vuillemin himself, belong to the genus *Monilia.*[11] For the fungus isolated once by Vuillemin, which has all the characteristics of the genus *Endomyces,* Landrieu has suggested the term *Endomyces vuillemini.*

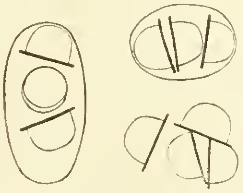

Fig. 26.—Willia ascospores.

Endomyces vuillemini Landrieu, 1912. *Synonym.*—*Endomyces albicans* Vuillemin, 1898.

This fungus was found by Vuillemin in 1898 in thrush patches.

Parasitic Life.—It forms white patches on the tongue and buccal mucosa. The patches are easily detached. A particle examined under the microscope shows septate mycelial threads, simple or ramified, the articles of which are straight or somewhat bent. Each article or cell is about 20 microns in length, and from 3 to 5 microns in breadth. At the terminal portion of each mycelial thread three or four shorter ovoid elements are found which reproduce by budding. Some similar ovoid or roundish globular refringent cells can be observed, originating laterally at the septations of the mycelium. These globular elements, which were at first considered to be spores, become detached, and reproduce by germination.

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[11] Castellani and Chalmers (footnote 3, p. 1079).

Saprophytic Life.—Culture Characters: The fungus grows well on slightly acid, Sabouraud's and other mediums, does not grow abundantly in alkaline mediums, does not ferment lactose and is gram-positive.

In cultures the fungus appears under two forms: (1) a filamentous form, showing the mycelial threads simple or ramified; (2) a globular form, morphologically similar to a typical yeast, and reproducing by budding. Both forms may be found in the same culture.

The fungus in cultures reproduces by: 1. Chlamydospores or external spores; large globular cells from 10 to 20 microns in diameter, with thick resistant walls. The chlamydospores are situated at the terminal extremity of some mycelial hyphae, and represent modified mycelial articles.

2. Asci containing four ascospores.

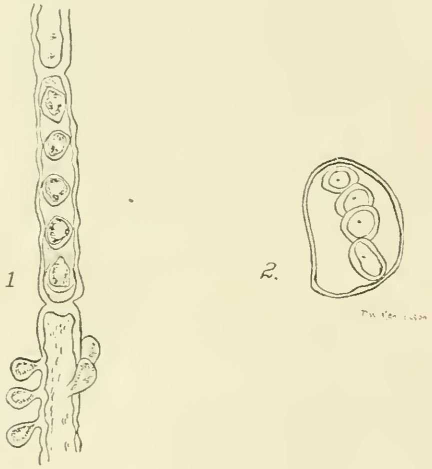

Fig. 27.—Endomyces vuillemini: (1) Mycelial thread showing arthrospores and conidia; (2) ascus with four ascospores.

3. Arthrospores. These are oval cells with membrane, protoplasm and nucleus similar to the external spores. The internal spores are arranged in a single string inside some mycelial tubes.

Pathogenicity.—The fungus was found by Vuillemin in a case of thrush, a condition generally due to fungi of the genus *Monilia.*[12]

Genus *Coccidioides* Rixford and Gilchrist, 1896.

Definition.—*Endomycetaceae* with mycelium well developed and asci containing a large number of spores, with intermediate characters between *Saccharomyces* and *Monilia.* One species only: *Coccidioides immitis* Rixford and Gilchrist, 1896.

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[12] Castellani, Aldo: Fungi and Fungous Diseases: Lecture II, Arch. Dermat. & Syph. to be published.

Coccidioides immitis Rixford and Gilchrist, 1896. *Synonyms.*—*Coccidioides pyogenes* Rixford and Gilchrist, 1896; *Oidium coccidioides* Ophüls, 1905; *O. protozoides* Ophüls, 1905; *Posadasia esferiformis* Canton, 1898; *Oidium immite* Verdun, 1907.

Definition.—*Coccidioides* with a large number of spores in the asci.

This organism was discovered by Wernicke in 1882 in America in a patient with patches resembling a tuberculide, and gummas. In the lesions are seen roundish bodies of various sizes, from 3 to 80 microns in diameter, with a well defined thick membrane. Inside some of the bodies numerous spores may be seen. This genus is not well defined.

Cultures.—The fungus grows under two types: (1) a saccharomyces-like type, reproducing by budding; (2) a filamentous type.

The colonies are roundish, slightly elevated, but deepening into the medium. In old cultures chlamydospores may be present. The biochemical properties of the fungus are not completely known; it does not cause gas fermentation in any sugar.

TABLE 8.—*Simple Classification of Yeastlike Fungi*

“Yeastlike” Fungi—Mycelium	Absent—Asci	Present, Saccharomyces sensu lato
		Absent, Cryptococcus sensu lato
	Present—Asci	Absent, Monilia sensu lato
		Present, Endomyces sensu lato

Pathogenicity.—The fungus induces in man a type of blastomycosis which has been thoroughly investigated in this country. It is pathogenic to monkeys, mice, guinea-pigs and rabbits. It probably lives saprophytic in nature.

SIMPLE SCHEME OF CLASSIFICATION OF “YEASTLIKE” FUNGI WHICH MIGHT BE USED BY CLINICIANS AND CLINICAL PATHOLOGISTS

The clinician and clinical pathologist do not require for their purposes in practice an elaborate classification as the pure mycologist does.

The term “yeastlike” fungi is somewhat unscientific but it is useful in practice to denote all fungi that reproduce solely or principally by budding and appear in the lesions under the shape of oval or roundish cells, some of them budding, with usually complete absence of mycelium; in cultures a little mycelium may be present, although the roundish or oval cells always greatly predominate.

The term "yeastlike" fungi, as generally understood at present by medical men, covers fungi of the following genera: *Cryptococcus, Coccidioides, Saccharomyces, Willia, Torula, Pityrosporum, Endomyces, Monilia, Mycoderma, Oidium*, as defined by certain authorities, and the doubtful genera *Parendomyces, Parasaccharomyces* and *Zymonema*.

The simple classification I have suggested is based on the presence or absence of mycelium and the presence or absence of ascospores.

SUBCLASS EUASCOMYCETES

Definition.—*Ascomycetes* with asci not separate or scattered, but approximate, usually forming a hymenium, and with a definite number of spores in an ascus.

Classification.—According to Stevens, the following orders may be recognized:

A. Asci approximate, no definite ascoma, but an inhymenium—*Protodiscales.*
B. Asci grouped in a definite ascoma.
    I. Asci not in a cylindric or globose perithecium—*Helvellales, Pezizales, Phacidiales, Hysteriales* and *Tuberales.*
    II. Asci in a cylindric or globose perithecium.
        (*a*) Perithecium sessile.
            1. Asci arising from a common level in the perithecium—*Perisporiales, Hypocriales, Dothidiales, Sphaeriales* and *Pyrenomycetes.*
            2. Asci arising at different levels in the perithecium—*Aspergillales.*
        (*b*) Perithecium on a short pedicle—*Laboulbeniales.*

Only the *Aspergillales* and the *Pyrenomycetes* interest us. The latter differ from the former by having their asci arranged in a hymenium within the closed ascocarp.

ORDER ASPERGILLALES

Definition.—*Euascomycetes* with asci gathered into definite cylindric or globose perithecia.

Type Family.—*Aspergillaceae.*

Classification.—The order contains a number of families, which may be recognized as follows. .

A. Peridium of loose floccose hyphae—*Gymnoascaceae* Baranetzky, 1872.
B. Peridium compact.
    I. Perithecia small.
        (*a*) Perithecia mostly sessile.
            1. Peridia closed—*Aspergillaceae.*
            2. Peridia open—*Trichocomaceae.*
        (*b*) Perithecia mostly stalked—*Oxygenaceae.*
    II. Perithecia large—*Elaphomycethaceae* and other families.

Only the *Gymnoascaceae* and the *Aspergillaceae* are of interest here.

Family *Gymnoascaceae* Baranetzky, 1872.

Definition.—Aspergillales with the peridium composed of loose hyphae, at the extremities of which the asci are situated, or without perithecial or ascal formation, and reproducing (as far as is known), as a rule, by mycelial or conidial spores.

Type Genus.—Gymnoascus Baranetzky, 1872.

Classification.—From the definition given previously, it is obvious that the family is capable of being divided into two tribes, as follows:

A. *Ascomycetes* type:

    With perithecia and asci—tribe 1, *Gymnoascaceae* Castellani and Chalmers, 1918.

B. *Fungi imperfecti* type:

    Without perithecia or asci; reproduction asexual by mycelial and conidial spores—tribe 2, *Trichophytoneae* Castellani and Chalmers, 1918.

Tribe *Trichophytoneae* Castellani and Chalmers, 1918.

Definition.—Gymnoascaceae of the *Fungi imperfecti* type.

Type Genus.—Trichophyton Malmsten, 1845.

Classification.—The various genera belonging to this tribe may be recognized as follows.

A. In lesions only mycelial filaments and no spores present—genus *Lophophyton* Matruchot and Dassonville, 1899.

B. In lesions mycelial filaments and spores present.

    I. In cultures no conidial-bearing hyphae found; do not attack hairs or hair follicles, but grow in the superficial or deep strata of the epidermis.

        (*a*) Pluriseptate spindles present in cultures; grow in the superficial strata of the epidermis, do not attack hairs; cultures not faviform—genus *Epidermophyton* Lang, 1879.

        (*b*) Pluriseptate spindles unknown in cultures; grow between the superficial and deep layers of the epidermis; cultures faviform—genus *Endodermophyton* Castellani, 1909.

    II. In cultures conidial-bearing hyphae present; may or may not attack hairs or hair follicles.

        (*a*) Conidia only at the ends of hyphae; large—genus *Montoyella* Castellani, 1907.

        (*b*) Conidia only at the sides of hyphae; not large—genus *Pinoyella* Castellani and Chalmers, 1918.

        (*c*) Conidia at the ends and sides of hyphae.

            1. In cultures fusiform bodies present as septate or nonseptate spindles; yellow favic scutula not present in lesions.

(A) Conidia on short stalks.
  (1) Attack hairs or hair follicles.
    (1^1) Grows in and on the surface of the hair; is often pyogenic and of animal origin—genus *Ectotrichophyton* Castellani and Chalmers, 1918.
    (2^1) Grows mainly in the hair, but a few mycelial filaments and spores can be found outside the hair; not pyogenic; of human origin—genus *Neotrichophyton* Castellani and Chalmers, 1918.
    (3^1) Grows entirely in the hair and filaments and spores cannot be found outside it; not pyogenic, except most rarely; of human origin—genus *Trichophyton* Malmsten, 1845.
  (2) Do not attack hairs or hair follicles—genus *Atrichophyton* Castellani and Chalmers, 1918.
(B) Conidia sessile—genus *Microsporum Gruby,* 1843.

2. In cultures fusiform bodies present in the form of swollen claviform ends of filaments, yellow favic scutula present in lesions—genus *Achorion* Remak, 1845.

Genus *Microsporum* Gruby, 1843.

Definition.—Gymnoascaceae with only *Fungi imperfecti* forms known; producing in the lesions mycelial filaments and mycelial spores, which are small, roundish, and about 2 to 3 microns in diameter. In cultures hyphae bearing sessile conidia and septate or nonseptate fusiform bodies may be seen. The lesions in man do not produce yellow favic scutula.

Type Species.—Microsporum audouini Gruby, 1843.

Remarks.—The fungi belonging to this genus are generally parasitic in the hairs and hair follicles, but may attack glabrous parts. When parasitic in the hairs, they are characterized by the mycelial spores being roundish, small (from 2 to 3 microns) and irregularly arranged in a mosaic-like manner. They are easily cultivated on Sabouraud's and other sugar mediums (see genus *Trichophyton*). In cultures the microsporons proliferate by sprouting with branching of the mycelium; and also, often, by:

1. Spore-Bearing Hyphae: of type *Acladium,* the conidia being oval, from 3 to 4 microns in length, and from 2 to 3 microns in breadth.

They are not supported by short sterigmas, as is the case in the genus *Trichophyton*.

2. Terminal Septate or Nonseptate Spindles (*Macroconidia*): These structures are found at the end of certain filaments, and may be considered to be modified terminal conidia. They are large fusiform structures, from 30 to 60 microns in length, and from 15 to 18 microns in breadth. They have granular contents, and may be septate or nonseptate. At the apex there are some peculiar hairlike formations which are not observed in the spindle of the fungi of the genus *Trichophyton*.

3. Chlamydospores: Certain mycelial articles become expansive, ovoid or ampulliform, from 22 to 18 microns in length, and 6 to 8 microns in breadth. Their protoplasm may be granular, and the membrane may become much thicker and have a double contour. These elements are chlamydospores. They are generally found when the fungi vegetate under unfavorable conditions. Other structures often seen in fungi of the genus *Microsporum* are the so-called denticulate or pectinate bodies, which are mycelial segments, generally curved, showing on one side—the convex side as a rule—several small protruding processes. Bodin has demonstrated that these processes are mycelial tubes arrested in their development.

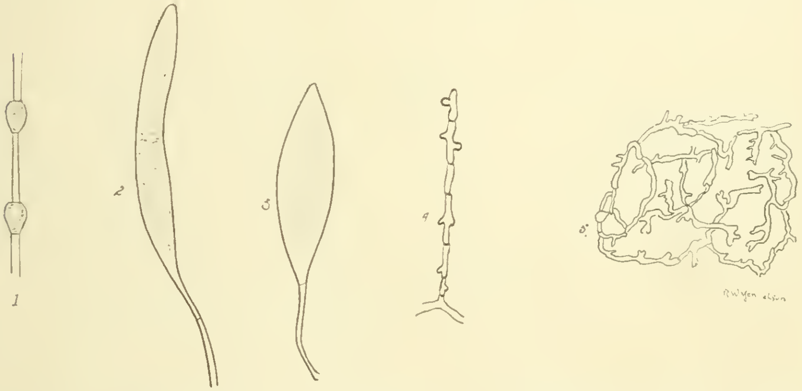

Fig. 28.—Microsporum audouini Gruby; (1) chlamydospores; (2) spindle (fusiform macroconidium); (3) pluriseptate spindle; (4) conidiophore (type Acladium); (5) denticulated structures (after Bodin).

Pleomorphism.—After a time, and often rapidly, the cultures of microsporons lose their characteristics and become pleomorphic. This phenomenon is not prominent, however, in the most important species, viz., *Microsporum audouini*, while it is accentuated in some microsporons of animal origin, especially *M. minimum*, in which, according to the complete investigation carried out by Bodin, two types may be encountered:

1. The downy type (*Acladium* type) is found on the ordinary sugar mediums in which there is an abundant development of white duvet. Microscopically, fructifications of the type *Acladium* are seen, the spore-bearing hyphae presenting numerous lateral sexile conidia. Subcultures will always give rise to the same downy type.

2. The glabrous type (*Endoconidium* type) is observed on wort agar. The growth has a smooth surface, without duvet. Microscopically, short strings of hyaline, ovoid or cylindric conidia are seen inside the terminal segments of the fertile hyphae. Lateral conidia are absent.

Table 9.—*Species of Microsporum Parasitic on Man*

	Genus	Species
Microsporum Gruby, 1843	Of human origin	M. audouini Gruby, 1843
		M. velveticum Sabouraud, 1907
		M. umbonatum Sabouraud, 1907
		M. ferrugineum Ota, 1921
	Of animal origin	M. tardum Sabouraud, 1909
		M. scorteum Priestley, 1914
		M. minimum Le Calvé et Malherbe, 1898
		M. lanosum Sabouraud, 1907
		M. felineum C. Fox and Blaxall, 1896
		M. fulvum Uriburu, 1907
		M. pubescens Sabouraud, 1909
		M. villosum Minne, 1907
		M. tomentosum Pelagatti, 1909
		M. iris Pasini, 1912
		M. flavescens Horta, 1912
		M. depauperatum Guéguen, 1912

Mode of Infection.—Infection takes place from man to man in the case of microsporons of human origin, and from the lower animals to man in the case of microsporons of animal origin. The human microsporons (*M. audouini, M. velveticum, M. umbonatum* and *M. tardum*) do not seem to be inoculable into the lower animals.

Some species (*M. audouini, M. velveticum, M. umbonatum* and *M. tardum*) seem to be parasitic on man only; others (*M. lanosum, M. felineum, M. minimum,* etc.) are parasitic on the lower animals, but occasionally infect man.

Microsporum audouini Gruby, 1843. This parasite was described by Gruby in 1843, but his investigation was forgotten, till Sabouraud, in his classic researches on ringworm in 1892, demonstrated the plurality of species of the fungi found in this condition, and showed that a form of tinea capitis was due to the microsporon described by Gruby.

Parasitic Life.—Around the affected hairs the fungus forms by means of its mycelial spores a white opaque sheath, extending 2 or 3 mm. above the opening of the hair follicle. The sheath is composed of a mosaic of small, roundish or polyhedral spores, the diameter of which varies between 2 and 3 microns. In the interior of the hair a few mycelial filaments of the fungus may be observed. The fungus seldom attacks glabrous parts of the body.

Saprophytic Life.—Cultures: *Microsporum audouini* grows well on Sabouraud's maltose agar and other mediums. The rate of growing is slow. In maltose agar the growth becomes evident about a week after inoculation under the appearance of a plaque of a so-called "satiny aspect," beneath the surface. In a few days more, aerial hyphae develop, extending above the surface. When the development is complete—generally this takes about six to eight weeks—the growth is roundish and covered with short grayish duvet, and presents often a central knob, and concentric whitish grayish rings The cultural characters, however, are variable, and pleomorphism occurs. The cultural characters have been thoroughly investigated by Sabouraud, Fox and Blaxall, Bodin and others. The fungus slowly liquefies gelatin; on potatoes it produces a brownish discoloration compared by Sabouraud to the color of dried blood.

Reproduction.—This takes place as follows:

1. By sprouts from the mycelial tubes.
2. By small terminal and lateral conidia, there being spore-bearing hyphae of type *Acladium.*
3. By large unilocular or multilocular spindles or fuseaux of French authors (macroconidia). These spindles are large structures from 30 to 60 microns in length and from 15 to 18 microns in breadth. They may be septate or nonseptate. The pole of the spindle often shows some delicate hairlike structures.
4. By formation of chlamydospores.

Pathogenicity.—*Microsporum audouini* causes the most obstinate form of tinea capitis. The patches affected are often large and scaly, and present stumps, provided with an opaque whitish sheath. It seldom attacks glabrous parts of the body. *M. audouini* is extremely common in England, but rare in the South of Europe (Italy), and extremely rare in the tropics. I have never seen cases of ringworm due to *M. audouini* in equatorial Africa or Ceylon, or in Louisiana, but a few cases have been described in Brazil, in Madagascar and in Senegal.

M. audouini seems to live only on the human subject, but closely allied species have been found by Fox in cats, by Bodin in dogs and by Bodin, Fox and others in horses.

Microsporum velveticum Sabouraud, 1907. Resembles closely *M. audouini*, but the cultures are more velvety, the duvet being white instead of grayish, and of closer growth; not inoculable into guinea-pigs. This species seems to be fairly common in North America.

Microsporum umbonatum Sabouraud, 1907. Found by Sabouraud in two cases of microsporosis of the scalp contracted in Russia. The appearance of the growth when it has reached complete development (about twenty-five to thirty days after inoculation) has been compared by Sabouraud to the appearance of an ancient round shield, with a central conical formation representing the umbo of the shield. It is not inoculable into guinea-pigs.

Microsporum tardum Sabouraud, 1909. Found by Sabouraud in cases of microsporosis capitis clinically identical with the type caused by *M. audouini;* differs from *M. audouini* by the growth being much slower and scantier, and the duvet being shorter.

Microsporum lanosum Sabouraud, 1907. *Synonym—Microsporon audouini* var. *canis* Bodin, 1897. Commonly found in dogs; may affect man, causing a type of severe tinea capitis; may also produce forms of tinea barbae and tinea corporis.

On Sabouraud's agar the growth is at first similar to *M. audouini,* only more abundant and more downy. Later, from twenty-five to thirty days after inoculation, the central portion of the growth becomes umbilicated, the depression being surrounded by a ring of snow-white duvet, which in old cultures may become yellowish. Pleomorphism is common.

Microsporum felineum C. Fox and Blaxall, 1896. Common in the cat in England, North America and Belgium; may infect man, attacking hairy and nonhairy regions of the body. On Sabouraud's agar the growth is rather abundant, discoid, with flattened surface, not showing any furrows and covered with a large amount of gray duvet. The fungus is easily inoculable into cats, dogs and guinea-pigs.

Microsporum minimum Le Calvé and Malherbe, 1898. *Synonyms—Microsporon audouini* var. *equinum* Bodin, 1896; *Trichophyton minimum* Le Calvé and Malherbe, 1898.

Common in fowls; may infect man, giving rise to a mild type of tinea corporis, in which cure is spontaneous.

On Sabouraud's and dextrose agar the growth is deeply furrowed, and in contrast to all other microsporons, duvet is absent, or, when present, is scanty, short and pinkish.

Microsporum fulvum Uriburú, 1907. Found in cases of tinea capitis in the Argentine. It grows rapidly on Sabouraud's agar, the growth presenting a central umbo, or projection, surrounded by a brownish powdery ring. The peripheral zone of the growth is covered with white duvet.

Microsporum pubescens Sabouraud, 1909. Discovered by Sabouraud in a case of tinea capitis contracted in New York. It grows rapidly on Sabouraud's medium, the culture being characterized by the presence of abundant but delicate silky duvet. At complete development, about thirty days after inoculation, the growth may have a flattened surface, or furrows, radiating from the center, may be present. The central portion, which is covered with rather long, white duvet, is encircled by a zone of powdery appearance; outside this there is the peripheral zone covered by the extremely delicate, characteristic, silky-like duvet.

Microsporum villosum Minne, 1907. Found in Belgium by Minne in a child suffering from ringworm of the hair clinically identical with the type caused by *M. audouini.* On Sabouraud's agar the growth at complete development is about 6 cm. in diameter; the central portion is flattened, with a powdery, slightly brownish surface. This is surrounded by mammillary, downy formations, which decrease in size toward the periphery.

Microsporum tomentosum Pelagatti, 1909. Found by Pelagatti in Sardinia in a case of microsporosis capitis, clinically identical with the usual type caused by *M. audouini*. It grows rapidly on Sabouraud's agar. The growth at complete development has a somewhat flattened appearance, but several furrows are present, originating from the central portion, which may be umbilicated. The whole surface is covered by thin white duvet.

Microsporum iris Pasini, 1911. Discovered by Pasini in Italy in some cases of microsporosis capitis, somewhat different clinically from the usual type. The fungus grows well on Sabouraud's agar. At complete development, from twenty-two to twenty-six days after inoculation, the growth presents a central knob covered with white duvet, and surrounded by white and brick-red rings, alternating.

The microsporosis capitis due to this microsporon is characterized by the hairs remaining nearly of normal length, and presenting a white greenish discoloration.

Microsporum flavescens P. Horta, 1912. Isolated by Horta from some circinate squamous patches situated on the neck of a child in Brazil; grows rapidly on Sabouraud's agar; the growth is yellow; there is a depression at the center, from which radiate four or five shallow furrows; pleomorphic duvet appears quickly.

Microsporum depauperatum F. Guéguen, 1912. Isolated by Guéguen from some circinate, dry, squamous patches. In cultures the membrane of some nucleal filaments presents peculiar thickenings. Spore-bearing hyphae are not so well differentiated as in other species.

Microsporum scorteum Priestley, 1914. This parasite resembles *M. fulvum* Uriburú, 1909, both morphologically and culturally, and may be identical therewith, as the descriptions given in Europe of Uriburú's parasite are scanty. It was found by Priestley in Townsville in tropical Queensland, and occurred in two circular inflamed areas on the calf of a boy's leg. There was not any scaliness, and the hairs were not visibly altered, though microscopically they contained mycelium and a few spores:

On Sabouraud's maltose agar the organism grew rapidly, producing a growth like a piece of chamois leather. It slowly digested milk, which it did not clot. Multiseptate spores were abundant, but lateral conidia were few, from 3 to 4 by 2 to 3 microns in size, while chlamydospores were infrequent. Nodular bodies like those found by Sabouraud in *T. lacticolor* were found. Spirals were frequent and pectinate bodies rare. Duvet was formed.

Microsporum ferrugineum Ota, 1921. *Synonym—Grubyella ferruginea* Ota. Growth on dextrose and maltose agars rusty red; common in Manchuria.

Genus *Trichophyton* Malmsten, 1845.

Definition.—Trichophytoneae with mycelial filaments and spores present in the lesions and conidial-bearing hyphae in cultures, only attacking hairs and entirely of human origin; seldom pyogenic.

Type Species.—Trichophyton tonsurans Malmsten, 1845.

General Considerations.—During their parasitic life the species of the genus *Trichophyton* vegetate according to two types: (1) mycelial filaments; (2) mycelial spores.

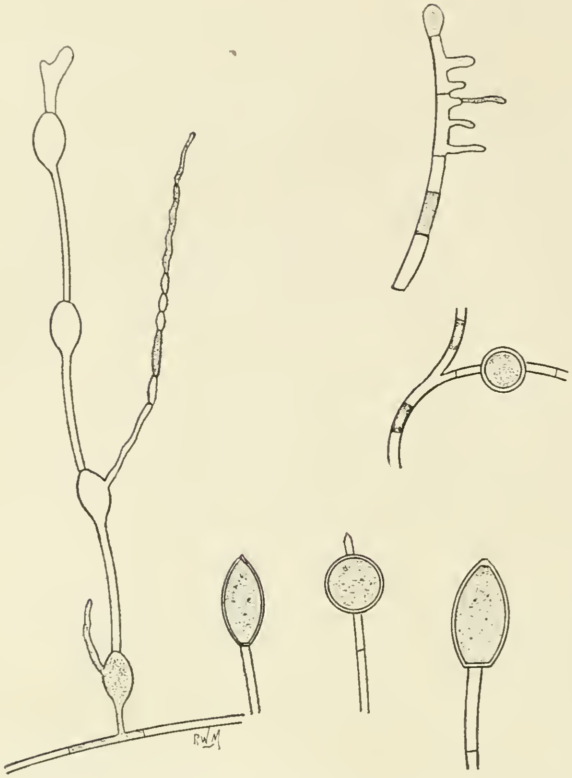

Fig. 29.—Microsporum ferrugineum Ota (after Ota and Langeron).

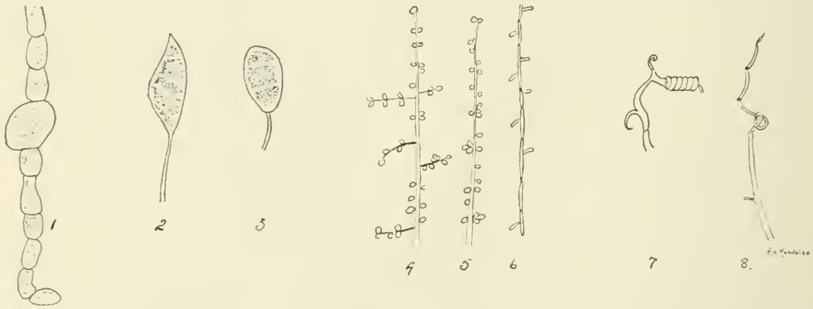

Fig. 30.—(1) Trichophyton—arthrospores and chlamydospore; (2) Trichophyton—septate spindle bodies; (3) Trichophyton—septate spindle bodies; (4) Trichophyton—spore bearing hyphae; (5) Trichophyton—spore bearing hyphae; (6) Microsporum—spore bearing hypha; (7) and (8) Trichophyton—spiral bodies.

The mycelial filaments consist of long cylindric cells, separated by septums. The so-called mycelial spores are simply a modification of the mycelial filaments, due to the septums being much closer, so that the cells limited by them are almost as broad as they are long. The term "mycelial spores" is incorrect, as they are not organs of reproduction, but only vegetative organs.

When the shape of these mycelial spores, or sporulating mycelia, or arthrospores is roundish or oval, the filament takes a moniliform appearance. Moreover, these cells are easily dissociated. Such a type is called "fragile mycelium." To this type belongs, for instance, *Trichophyton sabouraudi* R. Blanchard.

When the mycelial spores are square, the filament straight and its articles long, the mycelium is called "resistant." This type is observed, for example, in *Trichophyton tonsurans* Malmsten.

Cultivation.—Most trichophytons can be cultivated; some cannot. The best medium is Sabouraud's maltose agar, the composition of which is as follows:

Maltose	4.0 Gm.
Peptone Chassaing	1.0 Gm.
Agar	1.5 Gm.
Distilled water	100.0 cc.

On this medium, however, pleomorphism is of frequent occurrence.

Dextrose Agar.—Slightly acid dextrose agar 4 per cent is also good and has been used for many years by Pinoy, Sabouraud, Chalmers, me, and others.

Pollacci's medium has the following formula:

Broth (prepared by boiling 500 Gm. of meat finely triturated in 1,000 cc. of distilled water)	100.0 cc.
Peptone Witte	1.0 Gm.
Sodium chloride	0.5 Gm.
Agar	1.5 Gm.
Dextrose	7.0 Gm.

Pleomorphism.—Cultures on maltose and other sugar agars of all trichophytons, with the single exception of *T. sabouraudi,* as they grow old, lose their characteristics and become covered with abundant white duvet. In these cultures, which can be considered degenerate and are generally called "pleomorphic," organs of fructification are usually absent. By transplanting these cultures, one will obtain the same pleomorphic, downy type of growth; as a rule, the growth will never show the characteristics of the original young cultures obtained directly from the lesions. It is impossible to return to the original type, even by animal inoculations.

To prevent pleomorphism, Sabouraud advises the following medium:

Agar	1.8 Gm.
Peptone Chassaing	3 to 5.0 Gm.
Water	100.0 cc.

On this medium the growth of the various trichophytons is much less abundant than on sugar mediums, but the cultures are fairly characteristic, and they do not become pleomorphic.

Reproduction.—This takes place by:

1. Lateral and terminal conidia, supported by short sterigmas.
2. Chlamydospores; these are rare.
3. Large terminal septate and unseptate spindles. These spindles do not show at their free extremities the hairlike structures present in the spindles of the genus *Microsporum*.

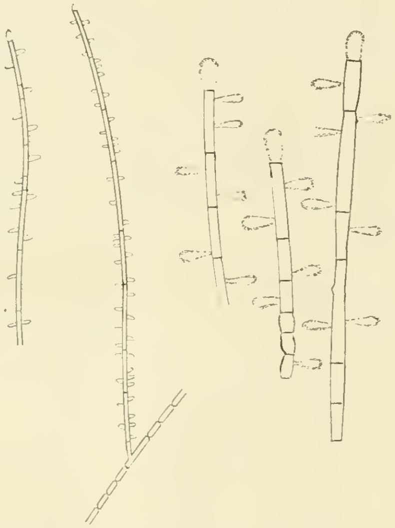

Fig. 31.—Trichophyton sabouraudi Blanchard (after Sabouraud).

Classification.—The principal species of the genus *Trichophyton* arranged chronologically are as follows:

1. *T. tonsurans* Malmsten, 1845.
2. *T. sabouraudi* R. Blanchard, 1895.
3. *T. violaceum* Bodin, 1902.
4. *T. sulphureum* C. Fox, 1908.
5. *T. glabrum* Sabouraud, 1909.
6. *T. fumatum* Sabouraud, 1909.
7. *T. effractum* Sabouraud, 1909.
8. *T. circonvolutum* Sabouraud, 1909.
9. *T. regulare* Sabouraud, 1909.

10. *T. umbilicatum* Sabouraud, 1909.
11. *T. exsiccatum* Uriburú, 1909.
12. *T. polygonum* Uriburú, 1909.
13. *T. soudanense* Joyeux, 1912.
14. *T. currii* Chalmers and Marshall, 1914.

These may be recognized as follows:

A. Condition of mycelium in hair not definitely stated, but probably that of the Crateriform subdivision as mentioned later.
    - (1) In cultures convoluted—*Circonvolutum.*

B. Condition of mycelium in hair definitely stated.
- I. Mycelium in hair resistant to caustic potash, segments characteristically quadrangular in shape, with double contour, from 4 to 6 microns in breadth, arranged in fairly straight ladder-like rows—Crateriform subdivision.
    - (*a*) Cultures colored and with craters—*Tonsurans* group.
        - (2) Yellow in center, white at periphery—*Tonsurans.*
        - (3) As *Tonsurans,* but, when old, cracked and dry—*Effractum.*
        - (4) Orange-red center, remainder sulphur colored—*Sulphureum.*
        - (5) Golden yellow convoluted center, becoming crateriform later—*Soudanense.*
        - (6) When old yellowish brown—*Fumatum.*
    - (*b*) Cultures white with craters—*Umbilicatum* group.
        - (7) Deeply umbilicated with aureola—*Umbilicatum.*
        - (8) Slow growth, surface cracked with dry appearance—*Exiccatum.*
        - (9) Growth at first roundish and then polygonal—*Polygonum.*
    - (*c*) Cultures white with knoblike center—*Currii* group.
        - (10) Does not form duvet—*Currii.*
- II. Mycelium in hair not resistant to caustic potash; segments rounded, 4 to 7 microns in diameter; not arranged, as a rule, in rows, but if a row is visible it resembles a string of beads and not a ladder—*Acuminate* subdivision.
    - (*a*) Without acuminate center—*Violaceum* group.
        - (11) Primary growth violet.
            - (A) Most strains do not melt gelatin rapidly.
                - (1^1) Ordinary amount of scaling on the head—*Violaceum.*
                - (2^1) Enormous numbers of scales, followed at times by permanent baldness—var. *Decalvans.*
            - (B) Melts strong gelatin rapidly, beginning as soon as growth appears—var. *Khartoumense.*
        - (12) Primary growth white—*Glabrum.*
    - (*b*) With acuminate center—*Sabouraudi* group.
        - (13) Without duvet when old—*Sabouraudi.*
        - (14) With duvet when old—*Pilosum.*

Genus *Neotrichophyton* Castellani and Chalmers, 1918.

Definition.—Trichophytineae with mycelium and spores present in the lesions, and conidia-bearing hyphae in cultures, attacking hairs, but with mycelial spores and filaments outside the hair shaft.

Type Species.—Neotrichophyton flavum Bodin, 1902.

Classification.—There are only two species, which may be distinguished as follows:

A. Cultures cerebriform—*Flavum.*

B. Cultures crateriform and creased—*Plicatile.*

Genus *Ectotrichophyton* Castellani and Chalmers, 1918.

Definition.—Trichophytoneae with mycelium and spores present in the lesions and conidia-bearing hyphae in cultures; attack hairs and hair follicles, growing in and on the surface of the hairs; often pyogenic and of animal origin.

Type Species.—Ectotrichophyton mentagrophytes Robin, 1853.

Classification.—The genus is capable of division into three subgenera by the following characters.

A. *Ectotrichophyton:*

> With small spores about 3 to 4 microns in diameter, forming a sheath outside the hair shaft, on dissociation of which they are seen to form chains; with sinuous and quadrangular hyphal segments, together with spores of varying diameter and air-bubbles, inside the hair shaft; with cultures easily obtainable, of rapid growth and of considerable vitality, characterized by plaster-like or floury centers surrounded by a fringe, when grown on Sabouraud's proof mediums, and by successful inoculations into animals—subgenus *Microtrichophyton.*

B. *Ectotrichophyton:*

> With large spores about 5 to 7 microns in diameter, forming a sheath outside the hair shaft, on dissociation of which they are seen to form chains, and with sinuous hyphal segments, together with large-sized spores and air-bubbles, inside the hair shaft; with cultures easily obtainable, but of slow growth in temperate climates, though much more rapid in tropical climates, characterized by their tendency to resemble (at all events when old) those of achorions, and capable of being inoculated into animals.
>
> I. With early formation of a duvet—subgenus *Ectotrichophyton.*
>
> II. Culture sooner or later resembles that of *Achorion schoenleini*—subgenus *Favotrichophyton.*

Ectotrichophyton (Favotrichophyton) Castellani and Chalmers, 1918.

Definition.—Ectotrichophyton with characters given previously for *Favotrichophyton.*

Type Species.—Ectotrichophyton discoides Sabouraud, 1909.

Classification.—Favotrichophyton species which are known are:

E. verrucosum Bodin, 1902.
E. ochraceum Sabouraud, 1909.
E. album Sabouraud, 1909.
E. luxurians Brault and Viguier, 1914.

These may be differentiated as follows:

A. Condition of mycelium in hair not definitely stated.
  I. Young cultures white, and soon resembling those of *Achorion schoenleini,* but sunk into the medium—*Album.*
B. Condition of mycelium in hair that of an ecto-endothrix.
  II. Cultures gray, humid, with verrucose surface—*Verrucosum.*
  III. Young cultures yellow ochre—*Ochraceum.*
  IV. Cultures yellowish brown or grayish yellow, cupola-shaped, humid with usually smooth surfaces, and not resembling *Achorion* cultures until old—*Discoides.*
  V. Rapid development—*Luxurians.*

Genus *Atrichophyton* Castellani and Chalmers, 1918.

Definition.—Trichophytoneae with mycelium and spores present in the lesions and conidia on short stalks, but they do not attack hairs.

Type Species.—Atrichophyton albiscicans Nieuwenhuis, 1907.

Classification.—The following table will indicate the characters of the species:

A. Has been cultivated.
  I. Cultures whitish with powdery surface—*Albiscicans.*
  II. Cultures brownish mass with deep furrows—*Macfadyeni.*
  III. Cultures pinkish with violet tinge—*Viannai.*
B. Has not been cultivated.
  I. Spores are numerous and of various sizes—*Blanchardi.*
  II. Spores are few and about 4 microns in diameter—*Ceylonense.*

INCERTAE SEDIS

Trichophyton balcaneum Castellani, 1916. Found in cases of peculiar condition of the scalp in the Balkans, which clinically resembled more a diffuse type of severe pityriasis sicca than a trichophytic condition.

When grown from scales on dextrose agar, it slowly formed a somewhat crinkled whitish growth. An interesting characteristic of this fungus is that apparently it does not become pleomorphic, even after numerous transplantations. Gelatin is rapidly liquefied. It does not form gas in any carbohydrate medium.

Microscopically the fungus shows features intermediate between a trichophyton *sensu lato,* a microsporum and an achorion.

Trichophyton louisianicum Castellani, 1926. Found by me in cases of tinea capitis and tinea corporis in New Orleans.

This classification of this fungus is somewhat difficult. For the time being I have placed it in the genus *Trichophyton*, section incertae sedis.

The growth is characteristic in dextrose agar; the central portion is covered with white duvet; the periphery is yellow or yellowish; the edge of the growth in fully developed cultures may be deep orange or tangerine. In oldish cultures in the submerged portion of the growth, dark red spots are not rarely seen.

The fungus can be separated from *T. sulphureum* found in England by the absence of crateriform colonies and of speckled appearance; from *Microsporum flavescens* by the absence of furrows originating from the central portion; moreover, *Microsporum flavescens* microscopically shows all the characteristics of the genus *Microsporum; T. ochraceum* is cerebriform and culturally, closely resembles *Achorion schoenleini; T. flavum* Bodin is typically cerebriform, so much so that Sabouraud called it *T. cerebriforme.*

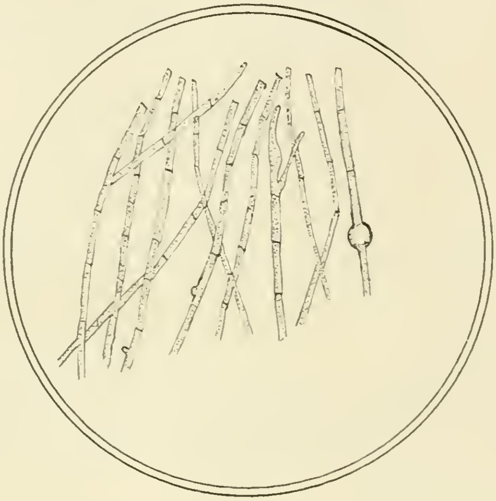

Fig. 32.—Trichophyton louisianicum (hanging-drop culture).

Genus *Achorion* Remak, 1845.

Definition.—Trichophytoneae with mycelial filaments and spores in the lesions; in cultures, conidial-bearing hyphae are present, with spores situated laterally and apically; fusiform bodies in cultures in the form of swollen claviform ends of filaments; yellow favic scutula are present in the lesions.

Type Species.—Achorion schoenleini Lebert, 1845.

Remarks.—The fungi belonging to this genus often, during parasitic life, show much longer mycelial segments than trichophytons and microsporons; masses of them, developing in hair follicles, form the well known sulphur-colored scutula which always develop round a hair. In the hairs, the mycelial tubes are frequently trichotomous and tetrachotomous, forming structures which have been compared in appearance to the skeleton of the human foot, and called "favus tarsi."

Cultures.—Sabouraud has noted that in hanging-drop cultures the spores sometimes develop slowly, sometimes rapidly. When the development is slow, the formation of numerous chlamydospores of various sizes, with a double contour membrane, takes place. When the development is rapid, one notes that the mycelium ramifies quickly in every direction, with the presence of few or no chlamydospores. The following structures may be noted:

1. *Claviform Bodies.*—The terminal portion of some filaments becomes swollen and claviform. These claviform filaments have been compared by some authorities to the "spindles" of the trichophytons and microsporons, but they are slenderer, and not septate. The French authors call these formations "chandeliers favique," because their shape somewhat resembles a candlestick.
2. *Favus Yellow Bodies.*—The protoplasm of some filaments collects itself at the terminal ends, the filaments becoming much thinner, and terminating in roundish or oval bodies, which must be considered terminal chlamydospores. It should be noted that in the typical achorions of human origin conidia-bearing hyphae are not seen, while these are present in the achorions of animal origin, in which separate spindles may be present.

Mediums.—Achorions grow well on all the usual sugar mediums, especially Sabouraud's agar and dextrose agar. They generally liquefy gelatin fairly rapidly.

Pleomorphism.—This is common in all achorions. If a culture becomes pleomorphic, it is impossible to make it revert to the original type.

Transmission.—In the case of achorions of human origin, infection takes place from man to man; in the case of achorions of animal origin, infection takes place from the lower animals. It is not impossible that achorions may live saprophytically in nature.

TABLE 10.—*Achorions*

Genus		
Achorion Remak, 1845	Of human origin (typical)	A. schoenleini Lebert, 1845
	Of animal origin (nontypical)	A. quinckeanum Zopf, 1890
		A. gypseum Bodin, 1907
		A. arloingi Blanchard, 1891

Achorions may be recognized as follows

A. Whitish yellow cerebriform colonies—*Schoenleini.*
B. White downy colonies—*Quinckeanum.*
C. Yellowish colonies—*Gypseum.*

There are several less known species, among which is *A. annulosum* Cayalbon, 1914.

A. arloingi has not been properly described.

Genus *Epidermophyton* Lang, 1879, emendavit Sabouraud, 1907.

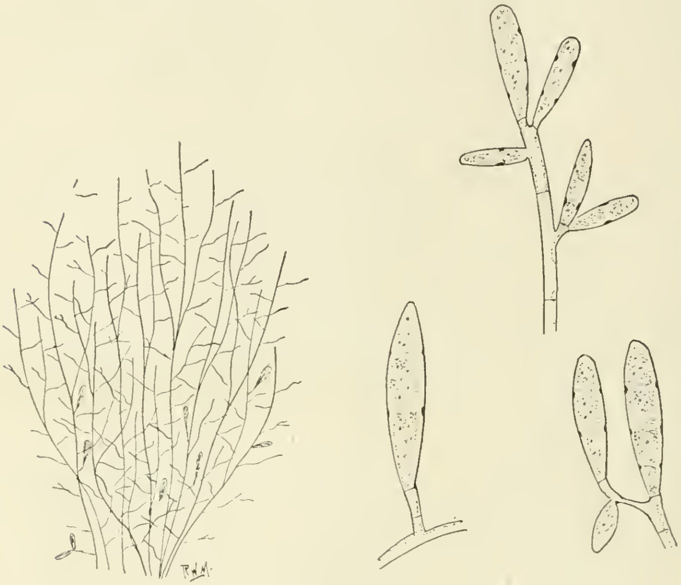

Fig. 33.—Epidermophyton cruris.

Definition.—Trichophytoneae with mycelial filaments and spores present in the lesions and with pluriseptate spindles present in the cultures; does not attack the hairs or hair follicles, but grows in the superficial layers of the epidermis.

Type Species.—Epidermophyton cruris Castellani, 1905.

Remarks.—The fungi belonging to this genus, which has been investigated by Sabouraud and me, grow superficially on the skin without invading the hairs and hair follicles; they do not produce suppuration. Reproduction takes place principally by pluriseptate spindles, with, on the average, from four to six cells. The septums, as noted by Pinoy, may not be complete, and the cavities may communicate. Spiral hyphae, as found in most species of *Trichophyton,* are absent; pectinate structures, as found in the microsporons, are absent;

spore-bearing hyphae, with lateral conidia of type *Acladium,* as noted both in the trichophytons and in the microsporons are not present. The cultures undergo rapid degenerative changes, losing their characteristics, and becoming covered with abundant, uniform, long, whitish duvet (pleomorphism). They are not inoculable into guinea-pigs, except Pinoy's *Epidermophyton simii.* The species so far known have been isolated from human lesions, except the epidermophyton discovered by Pinoy in monkeys.

TABLE 11.—*Epidermophytons*

Genus	Principal Species
Epidermophyton Lang, 1879, emendavit Sabouraud, 1907	E. cruris Castellani, 1905 E. perneti Castellani, 1907 E. rubrum Castellani, 1907

These species may be recognized by their growths on Sabouraud's agar as follows:

A. Peculiar light yellow (lemon yellow)—*Cruris.*
B. Pinkish—*Perneti.*
C. Deep red—*Rubrum.*

Less known species are *E. interdigitale* Priestly, 1917; *E. plurizoniforme* McCarthy, 1925; *E. lanoroseum* McCarthy, 1925; *E. niveum* McCarthy, 1925, and *E. salmoneum* de Mello.

For *E. simii* Pinoy, 1911, the genus *Pinoyella* has been created.

Genus *Endodermophyton* Castellani, 1909.

Definition.—*Trichophytoneae* with mycelial filaments and spores in the lesions, but no conidial filaments in cultures; pluriseptate spindles unknown; grows between the superficial and deep layers of the epidermis, and does not attack the hair or hair follicles.

Remarks.—The fungi belonging to this genus are characterized by their growth between the superficial and deep strata of the epidermis, forming an interlacing felt of mycelia, which detaches the horny and granular layers from the rete Malpighi. They do not invade the hair follicles and do not cause suppuration. They have been cultivated by me.

Cultures.—Botanically, these fungi, in the appearance of the cultures, are closely allied to achorions, as remarked by Sabouraud and Pinoy, who have examined my cultures. Attempts at cultivation failed for a long time, as these fungi generally do not grow on solid mediums direct from the scales. These, after being treated with alcohol for from five to ten minutes, must be placed in dextrose-broth tubes, one scale in each tube.

TABLE 12.—*Fermentative Characters of Certain Monilias and Bacteria* *

	Dextrose	Levulose	Maltose	Galactose	Saccharose	Lactose	Mannitol	Dulcitol	Dextrin	Raffinose	Arabinose	Adonitol	Inulin	Sorbitol	Starch	Glycerol	Inositol	Salicin	Amygdalin	Isodulcitol	Erythrytol
Monilia balcanica Castellani	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia krusei Castellani	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia pinoyi Castellani	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia metalondinensis Castellani	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia tropicalis Castellani	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia metalondinensis rhoi Castellani	+	+	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia bronchialis Castellani	+	+	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia macedoniensis (M. macedoniensoides) Castellani	+	+	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacillus coli-communis Escherich, strain 0	+	+	+	+	0	+	+	+	+	+	+	0	0	+	0	+	0	+	0	+	0
Bacillus coli-communior, strain 0 (pseudocoscoroba)	+	+	+	+	+	+	+	+	+	+	+	0	0	+	0	+	0	+	0	+	0
Bacillus paratyphosus B Schottmüller, var. M	+	+	+	+	0	0	+	+	+	0	+	0	0	+	0	0	+	0	0	+	0
Bacillus paratyphosus A Schottmüller	+	+	+	+	0	0	+	+	+	0	+	0	0	+	0	0	0	0	0	+	0
Bacillus columbensis Castellani	+	+	+	+	0	0	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0
Bacillus asiaticus Castellani	+	+	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	0	0	+	0
Bacillus pseudo-asiaticus Castellani	+	+	+	+	+	0	+	+	+	+	+	0	0	+	0	+	0	+	0	+	0
Bacillus morgani Castellani and Chalmers, strain S	+	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacillus orleanensis Castellani	+	+	+	+	+	+	+	+	+	+	+	0	0	+	+	0	0	+	+	+	0
Monilia pseudotropicalis IV Castellani	+	+	0	+	+	+	0	0	0	+	0	0	+	0	0	0	0	0	0	0	0

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* In this and the following tables and in the mycologic formulas, + indicates gas; 0, absence of gas; simple acid fermentation is not taken into account.

Most of the tubes become contaminated with bacteria, but in those which remain clear, after a time (from five to ten days), a few delicate, short, white filaments will be seen originating from the scale. The growth slowly increases until, after from three to four weeks, it takes the appearance of a small, white, fluffy mass, with a dark spot (the scale) in the center. Portions of the broth cultures are sown on solid sugar mediums, on which growth takes place easily at that time. Fungi can then be indefinitely subcultured on solid mediums. The fungi grow much more abundantly on dextrose agar 4 per cent, than on Sabouraud's or any other mediums.

Reproduction.—In hanging-drop cultures, long mycelial filaments are seen; conidia-bearing hyphae are not present; reproduction occurs apparently by sprouting, branching taking place; but further investigation is necessary on the subject.

Pleomorphism.—Pleomorphism is much less marked than in the trichophytons, epidermophytons and achorions, but old cultures may lose their characteristics, becoming covered with duvet.

Endodermophytons are found in man in the following order of frequency:

E. tropicale Castellani.
E. indicum Castellani.
E. concentricum Blanchard.
E. mansoni Castellani.

These may be recognized as follows:

A. Dextrose agar cultures amber colored, no or only slight duvet—*Tropicale.*
B. Dextrose agar cultures red.
    I. Causes tinea imbricata—*Indicum.*
    II. Causes tinea intersecta—*Castellanii.*
C. Dextrose agar cultures after a time black.
    I. Black pigmentation fairly slow—*Concentricum.*
    II. Pigmentation very rapid—*Mansoni.*

Genus *Pinoyella* Castellani and Chalmers, 1908.

Definition.—Trichophytoneae with mycelial filaments and spores in the lesions, and in cultures conidia-bearing hyphae, with the spores situated laterally only.

Type and Only Species.—Pinoyella simii Pinoy, 1911; *Epidermophyton simii* Pinoy, 1911.

The fungus was discovered by Pinoy in a trichophytic-like eruption observed on a monkey. On Sabouraud's agar, the growth is at first yellowish orange, somewhat similar to *Epidermophyton cruris;* later, the growth is whitish and covered with white duvet.

Pinoy's fungus has several interesting botanic features, some of which are those of the genera *Microsporum* and *Trichophyton.* In contrast to the typical epidermophytons, it presents spore-bearing hyphae with lateral conidia, and is inoculable into guinea-pigs, in which it produces a trichophytic-like eruption.

Ota and Langeron have devised a new classification of the fungi belonging to the genera *Trichophyton, Microsporum, Epidermophyton* and *Achorion.* They emphasize the point that the usual classification of these fungi is based, to a great extent, on the different pathologic lesions they produce rather than on purely botanical characters. They consider all these fungi, except *T. currii* Chalmers and Marshall, 1914, to belong to the order *Hyphomyceti,* family *Conidiosporeaceae,* subfamily *Closterosporeae* (new subfamily), and separate them into the following genera:

Trichophyton Malmsten, 1848, emendavit Ota and Langeron, 1923.

Sabouraudites Ota and Langeron, 1923.

Bodinia Ota and Langeron, 1923.

Endodermophyton Castellani, 1909.

Grubyella Ota and Langeron, 1923.

Epidermophyton Lang, 1879, emendavit Ota and Langeron, 1923.

In the foregoing classification the genera *Microsporum* and *Achorion* disappear. For the old *Trichophyton currii* Chalmers and Marshall, which produces rudimental perithecia they have created the genus "*Atcleothylax*" Ota and Langeron (family *Gymnoascaceae,* order *Ascomyceti*).

These genera may be recognized as follows:

A. Ascospores and perithecia absent.

I. Aleurospores (lateral conidia) present in large numbers, "fuseaux" (terminal pleuriseptate macroconidia) absent or scanty, denticulated and spiral structures absent—*Trichophyton.*

II. Aleurospores (lateral conidia) numerous, "fuseaux" present, denticulated and spiral structures present—*Sabouraudites.*

III. Aleurospores (lateral conidia) absent or rudimentary, "fuseaux" numerous—*Epidermophyton.*

IV. Aleurospores (lateral conidia) absent or rudimentary, "fuseaux" absent, chlamydospores and claviform bodies often well marked and numerous—*Grubyella.*

V. Aleurospores (lateral conidia) absent or rudimentary "fuseaux" absent, arthrospores present in small chains—*Bodinia.*

VI. Same as in part V, but arthrospores not so well defined—*Endodermophyton.*

B. Rudimentary perithecia present—*Atcleothylax.*

From the recent investigation of Pollacci and Nannizzi it would appear that a number of fungi of the genus *Trichophyton,* etc., may produce perithecia and ascospores after prolonged cultivation on special mediums such as bird's feathers; this would invalidate to a great extent Ota and Langeron's new classification.

Family *Aspergillaceae.*

Definition.—Aspergillales with compact peridium, small sessile closed perithecia.

Type Genus.—Aspergillus Micheli, 1729.

Classification.—The genera of *Aspergillaceae* can be recognized as follows:

- A. Spores unicellular.
    - I. Perithecium beaked—*Microascus.*
    - II. Perithecium not beaked.
        - (*a*) Perithecia with appendages—*Cephalotheca.*
        - (*b*) Perithecia without appendages.
            - 1. Conidiophores absent—*Thelavia.*
            - 2. Conidiophores present.
                - (A) Conidia solitary—*Aphanoascus.*
                - (B) Conidia in chains.
                    - (1) Conidiophores simple—*Emericella.*
                    - (2) Conidiophores enlarged apically and bearing sterigmas.
                        - (1^1) Sterigmas simple—*Aspergillus.*
                        - (2^1) Sterigmas branched—*Sterigmatocystis.*
                    - (3) Conidiophores branched.
                        - (1^1) Sympodially branched—*Eurotiopsis.*
                        - (2^1) Bushy branched.
                            - (a^1) In bundles, perithecia stalked— *Penicililopsis.*
                            - (b^1) Not in bundles, perithecia sessile—*Penicillium.*
- B. Spores bicellular—*Testudina.*

One is concerned with the genera *Penicillium, Aspergillus* and *Sterigmatocystis.*

Genus *Penicillium* Link, 1809.

The whole fruit-bearing hypha with its sterigmas and conidia resembles a hair pencil, hence the name of the genus (*Penicillium* = hair pencil). The conidiophore hypha shows verticillate branches, which give rise to slender fusiform formations (sterigmas) abstricting chains of conidia.

The following species have been found parasitic in man:

P. crustaceum Linnaeus, 1763.
P. minimum Siebenmann, 1889.
P. barbae Castellani, 1907.
P. montoyai Castellani, 1907.
P. pruriosum Salisbury.
P. brevicaule—var. *hominis*—Brumpt and Langeron, 1910.

P. crustaceum: bronze spores about 4 microns in diameter.

P. minimum: spores from 2½ to 3 microns, brownish black.

P. montoyai: spores from 3 to 4½ microns, dark grayish.

P. brevicaule: spores spherical, occasionally oval, chocolate colored.

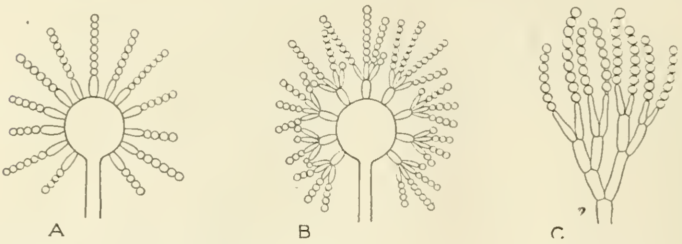

Fig. 34.—*A*, Aspergillus; *B*, Sterigmatocystis; *C*, Penicillium. After Brumpt.)

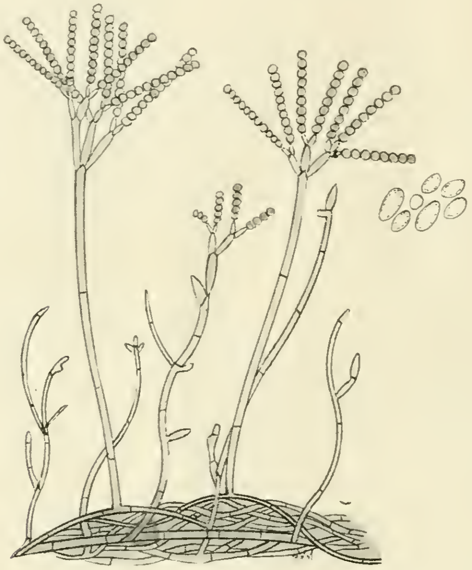

Fig. 35.—Penicillium crustaceum.

P. barbae: covers probably several species; grows on hairs of moustache and beard, producing small, grayish or dark grayish and even black nodules.

P. pruriosum: found by Salisbury in a case of pruritus vaginae.

Genus *Aspergillus* Micheli, 1725.

The conidiophore hyphae are not ramified, and they terminate into ovoid or roundish formations, which support numerous claviform elements (sterigmas), each of which supports a chain of roundish conidia.

Aspergilli are generally saprophytes, but they may become parasites. They are easily grown on acid mediums, liquid or solid, and also on alkaline mediums. Iron and manganese have a favorable influence on the growth and sporulation of these fungi.

A medium much used is Raulin's liquid, the formula of which is: cane sugar, 70 Gm.; tartaric acid, 4 Gm.; ammonium nitrate, 4 Gm.; ammonium phosphate, 0.6 Gm.; potassium carbonate, 0.6 Gm.; magnesium carbonate, 0.4 Gm.; ammonium sulphate, 0.25 Gm.; zinc sulphate, 0.07 Gm.; ferrous sulphate, 0.07 Gm.; potassium silicate, 0.07 Gm.

Fungi of the genus *Aspergillus*, when growing parasitically in the tissues, often lose their characteristics; the typical fructifications are absent, and only mycelial threads and roundish or oval yeastlike bodies are seen.

The principal species may be differentiated with some difficulty as follows:

A. Green species:
    I. Spores 5 microns and more; can hardly be differentiated—*Repens, Flavus.*
    II. Spores less than 5 microns.
        (*a*) Lives saprophytically; green on acid mediums, brownish black on alkaline or neutral mediums.—*Fumigatus.*
        (*b*) Not known to live saprophytically.
            1. Similar to *Fumigatus* but conidial chains long—*Jeanselmei.*
            2. Pathogenic for rabbits—*Malignus.*
            3. Found in bronchial sputum—*Bronchialis.*
B. Blackish brown species—*Nigrescens.*
C. Golden, brownish, gold-brown or reddish—*Herbariorum.*

Genus *Sterigmatocystis* Cramer, 1859.

Definition.—Aspergillales with conidiophores terminating in roundish or ovoid formations, on which are situated short cylindric structures—primary sterigmas—surmounted by similar elements—secondary sterigmas. Each of the secondary sterigmas supports a chain of roundish conidia.

Remarks.—The genus was created by Cramer for a fungus found in a man's ear.

Type Species.—Sterigmatocystis antacustica Cramer, 1859.

Classification.—The two species of importance can be recognized as follows:

A. Young conidial forms green; later strongly gray to brown; conidia small, 3 microns in diameter—*Nidulans.*

B. Conidial forms blackish brown; conidia small, 2.5 microns in diameter—*Antacustica.*

(To be continued)

FUNGI AND FUNGOUS DISEASES

LECTURE I *

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NEW ORLEANS

(Continued from page 604)

CLASS III. PHYCOMYCETES

The *Phycomycetes*, which are characterized by the mycelium being continuous and nonseptate, in the vegetative stage, contain several families, one of which, *Mucoraceae*, has organisms parasitic of man. The fungi belonging to this family have a branching mycelium with aerial branches (gonidiophores), each of which suports on its distal extremity a globular, pear-shaped or claviform sporangium, called gonidangium, which is at first separated from the gonidophore by a septum. This septum later protrudes into the lower portion of the sporangium and forms a variously shaped structure known as columella. By free cell formation, gonidia develop inside the sporangium. The sporangial protoplasm not used in the formation of the gonidia is transformed into a peculiar mucillaginous substance, which later dries up and is the cause of the sporangium bursting. Each gonidium which becomes free gives rise by germination to a mycelial filament. There is also at times a sexual form of reproduction which consists in the conjugation of undifferentiated nonmotile gametes, with the formation of zygospores. When growing in unfavorable mediums numerous species reproduce only by conidia and chlamydospores.

The family *Mucoraceae* contains a number of genera, among which the following have species parasitic of man:

1. *Mucor* Micheli, 1729. Mycelium ramified, rhizoids absent.
2. *Rhizomucor* Lucet and Costantin, 1900. Rhizoids (slender rootlike filaments) present, columella ovoid.
3. *Rhizopus* Ehrenberg, 1820. Rhizoids present, columella mushroom-like.
4. *Lichtheimia* Vuillemin, 1904. Peduncle supporting sporangium ends in a special formation encircling the base of the columella.

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* The Adolph Gehrmann Lectures of the University of Illinois College of Medicine, 1926.

For a more complete classification of the *Phycomycetes,* the reader is referred to the "Manual of Tropical Medicine" by Castellani and Chalmers.[13]

SIMPLE CLASSIFICATION OF FILAMENTOUS FUNGI INCLUDING MOLDS FOR THE USE OF CLINICIANS AND CLINICAL PATHOLOGISTS

I will present here a simple scheme for the classification of filamentous fungi, just as I did in the case of so-called yeastlike fungi.

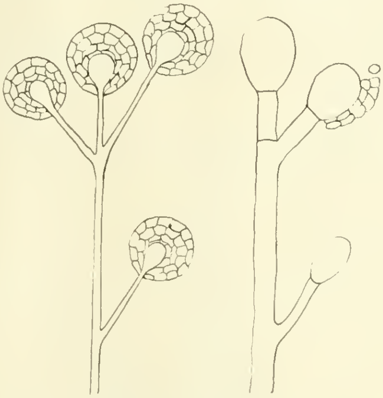

Fig. 36.—Rhizomucor parasiticus Lucet and Costantin.

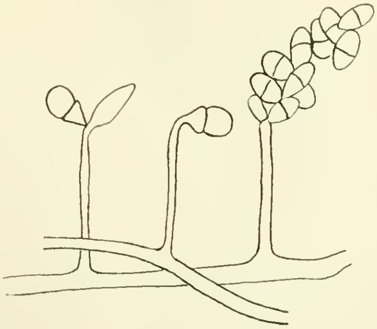

Fig. 37.—Trichotecium (from Buchanan).

Filamentous fungi, viz., fungi with abundant mycelial filaments, while free yeastlike or budding cells are scanty or absent, may from a practical point of view, be separated into two large groups:

Group A: well defined fructifications absent, cultures usually not fluffy, *Nocardia-oidium* group *sensu lato.*

Group B: well defined fructifications or specially shaped conidia present, cultures often fluffy, molds group *sensu lato.*

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[13] Castellani and Chalmers (footnote 3, p. 972).

Group A may be divided into two subgroups:

1. Mycelial threads thin, bacillus-like—*Nocardia sensu lato* (comprises *Nocardia, Cohnistreptothrix, Leptothrix, Vibriothrix*.
2. Mycelial threads of larger dimensions, often closely septated—*Oidium sensu lato* (comprises *Oidium sensu stricto, Trichosporum, Madurella, Indiella hemispora*).

Group B, which is characterized by mycelial threads of a fairly large size, cultures that are often fluffy and the presence frequently of distinctive fructifications, comprises those fungi which in clinical laboratories are known as molds. It comprises also the *Trichophyton, Microsporum, Achorion, Epidermophyton, Endodermophyton* group of fungi, the cultures of which, especially when old, have some similarity to ordinary molds.

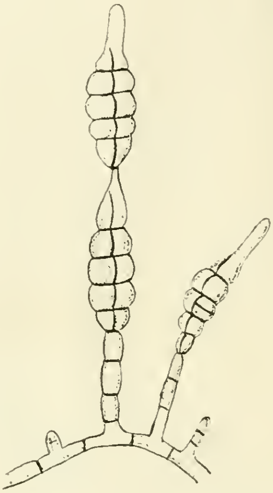

Fig. 38.—Alternaria (from Buchanan).

Group B (molds *sensu lato*) may be classified as follows:

1. Isolated from ringworm or ringworm-like conditions; large spindle-like terminal structures (fuseaux) often found in cultures—*Trichophyton sensu lato* (comprises *Trichophyton, Microsporum, Epidermophyton, Achorion* and *Endodermophyton*).
2. Often *Saprophytes*, and when pathogenic do not as a rule cause ringworm-like lesions; fuseau absent; distinctive fructifications often ringworm-like lesions; fuseaux absent; distinctive fructifications often present—molds *sensu stricto*.

The fungi that are known as molds in clinical laboratories (*Aspergillus, Penicillium, Mucor*, etc.) belong to different classes of fungi—*Ascomycetes, Phycomycetes*, etc., of which I have given a botanic description in the preceding pages. For practical purposes they may be described as characterized by a much branched mycelium and growing on ordinary laboratory mediums and also on decaying material, as a cottony, fluffy or velvety mass.

Buchanan gives the following useful key, based on the characters of the conidia (asexual spores), for the recognition of the nine most common genera.

Key for Differentiation of Common Genera of Molds

A. Spores borne in sporangia. Mycelium nonseptate.
    B. Sporangiophores arising in clusters from the nodes of runners or stolons....................1. *Rhizopus*
    BB. Sporangiophores arising singly, without stolons..2. *Mucor*

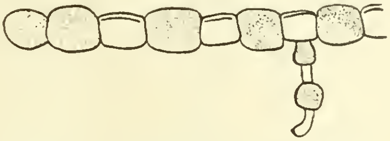

Fig. 39.—Chlamydospores of Mucor.

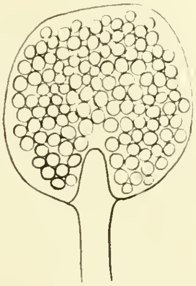

Fig. 40.—Sporangium of Mucor.

AA. Spores (conidia) never borne in sporangia.
    B. Neither conidia nor hyphae smoky or dark in color.
        C. Conidia 1 celled.
            D. No distinct conidiophores. Conidia formed as oidia..........3. *Oöspora*
            DD. Conidia on distinct conidiophores.
                E. Conidiophores enlarged or club-shaped at tip...................4. *Aspergillus*
                EE. Conidiophores branched at tip....5. *Penicillium*
        CC. Conidia two-celled, pear-shaped ....6. *Trichothecium*
    BB. Either conidia or hyphae smoky or dark, or both.

C. Conidia one-celled, in branched chain..........7. *Hormodendrum*

CC. Conidia more than one-celled.

D. Like *Hormodendrum*, but conidia two-celled when old....................................8. *Cladosporium*

DD. Spores many-celled, club-shaped, in chains....9. *Alternaria*

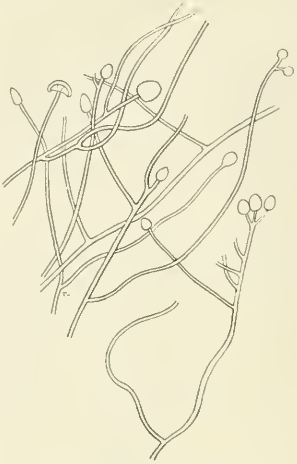

Fig. 41.—Rhizomucor septatus Von Bezold (after Siebemann).

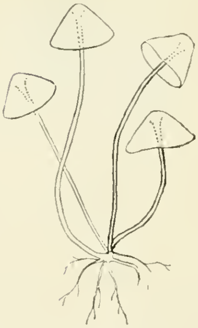

Fig. 42.—Rhizopus niger Ciaglinski and Hewelke.

BIOLOGIC CHARACTERS OF FUNGI

I will now pass from the morphologic characters of fungi to a more interesting subject: their biologic and biochemical characteristics. The more one studies the fungi, the more one sees how closely related they are to true bacteria with regard to biologic and biochemical characters.

Agglutinins.—Twenty-five years ago the late Dr. Macfayden, director of the Lister Institute, inoculated rabbits with cultures of a saccharomyces, and observed a production of specific agglutinins in the blood for the particular species he had inoculated. These results were confirmed and enlarged, using other fungi, by G. H. Rogers, Concetti, Quarelli and others, and Widal and Abrami have described a general diagnostic method, "Sporo-agglutination Method," based on the observation that in patients suffering from mycologic diseases are found specific agglutinins for the spores of the causative fungi. In my experience, however, in many cases, in addition to specific agglutinins, there is present also a large amount of nonspecific agglutinins, and this generally detracts from the usefulness of the method.

Toxins.—Fungi may produce powerful toxins. Some years ago, Auchair and Verliac obtained a toxic substance soluble in ether from *Nocardia bovis*, and they called it "Actinomycetine." Charrier, Roger and others have succeeded in extracting toxins from monilia fungi. Various toxins have also been extracted from fungi of the genus *Aspergillus*, and at one time a number of authorities, including Ceni, Besta and others, believed that pellagra was caused by these aspergillus toxins.

Vaccines.—Mycetic vaccines have been prepared by various authorities. Plato and others prepared a trichophyton and microsporal vaccine by killing cultures of different trichophytons and microsporons by heat, and then triturating the mass. By using vaccines intradermally various cutireactions have been devised; for instance, De Beurmann devised a sporotricho-cutireaction for the diagnosis of sporotrichosis; others have devised a trichophitic cutireaction for the diagnosis of atypical cases of ringworm, etc.

Biochemical Changes Induced by Fungi.—Certain fungi may induce profound chemical changes and play an extremely important rôle in nature and are of great importance in agriculture and industry. Certain fungi are intimately connected with the metabolism, life history and successful development of the higher plants. Mycelial fungi grow on and in the roots of a large number of these plants, and it is probable that in some cases nitrogen is furnished to the growing plants by these organisms. The rôle played by organisms of the family *Saccharomycetaceae* with regard to alcoholic fermentation, and the production of wine, beer, cider and other fermented beverages, is well known.

Fungi are of great utility in the ripening of cheese, and the flavor of each particular cheese is to a great extent due to the different fungi present. For instance, *Penicillium roquefortii* gives the characteristic taste of Roquefort cheese. The odor and, to a great extent, the taste of Camembert cheese is caused by *Penicillium camembertii*, in addition to other molds, such as *Oidium lactis*.

SYMBIOSIS

A number of fungi are capable of living in symbiosis. The subject of symbiosis has so far attracted little attention from medical men. I use of the term symbiosis *sensu lato* to indicate the living together of two organisms in close association, their close association not being detrimental to either of them; symbiosis *sensu stricto* means that the association not only is not detrimental, but also is mutually beneficial. Botanists years ago discovered that a large group of plants—the lichens—represent a symbiosis of a fungus and an alga; they also found that symbiosis plays an important rôle in the nutrition process of numerous plants and in the germination of certain seeds. I shall deal briefly with symbiosis in general, as so little on the subject can be found in medical books.

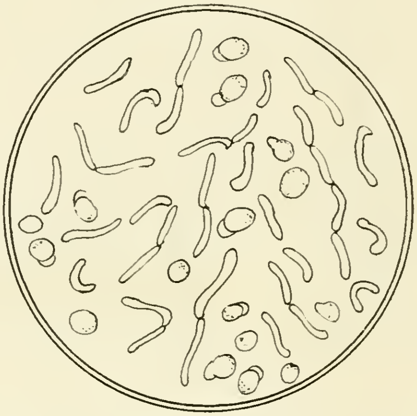

Fig. 43.—Cryptococcus graciloides and Bacillus vermiculoides living in symbiosis.

Symbiosis Between Two Vegetal Organisms.—Composite Plants: The best known example is furnished by the lichens. A lichen is a composite plant consisting of a green alga and a fungus which may be grown separately with difficulty. Every one has observed the green dust that often occurs on lichens. This powdery dust consists of young associations called soredia of algal cells and fungal hyphae. Another well known symbiotic plant is the ginger-beer plant, the lumps of which are composed of a yeast (*Saccharomyces pyriformis*) and a bacterium (*Bacterium vermiforme*).

Symbiosis Between a Vegetal Organism and an Animal Organism.—Symbiosis may also occur between plant and animal. For instance, in the marine worm convoluta an alga is always present, and in the larvae of certain aphids, coccids, etc., yeasts occur as more or less definite structures.

Importance of Symbiosis in the Nutrition Processes of Certain Plants.—Reissek, in 1847, came to the conclusion that fungi were normally present within the cortical cells of the roots of various flowering plants, being best developed in the underground roots of orchids. Soon after, another type of association was noted, especially in forest trees. Here the fungus mycelium forms a sort of mantle around the root, in contrast to being within the cells of the cortex. This association is common in Castanea and Corylus. The rootlets so infected generally take a characteristic coralloid appearance. Frank, in 1885, coined the term "mycorrhiza" to denote fungi (including bacteria) in or around the roots of plants. He used the term "endotrophic mycorrhiza" for those forms in which the fungus occurred within the tissues of the host, and the term "ectotrophic mycorrhiza" when the fungal mycelium surrounded the rootlets as a sheath.

The antiquity of such associations is seen in the fact that they occur in fossil plants such as *Rhynia*, *Hornea* and *Asteroxylon* from the lower (or middle) Devonian period. Weiss, in 1906, recorded mycorrhiza in fossil roots from the lower coal measures, for which he suggested the name "mycorrhizonium."

A well known instance of symbiosis is represented by the nodules found in the roots of many leguminous plants. For many years it was noted that roots of leguminous plants, such as peas and clover, presented a large number of nodules or tubercles which were for a long time considered to be normal morphologic features of such plants. Later it was found that these nodules are formed by the presence of a bacillus (*B. radicola*). This bacillus is apparently always present in the soil; the bacillus enters through a root-hair, it reaches the interior of the cortex and begins multiplying there, producing the nodular outgrowths. The organism feeds and grows principally on the carbohydrates (sugar, etc.) supplied by the host plant, these having been produced as the result of the photosynthetic activity of plant leaves. The bacillus is therefore supplied by the plant with carbohydrate food, but the bacillus in return supplies the plant with the nitrogenous compounds necessary for the production of protoplasm, by utilizing the free nitrogen of the air circulating in the soil.

Most plants have to take their nitrogen in a combined form, as ammonia salts, nitrates, etc., as nitrogen is an inert element and difficult to force into combination with others. *B. radicola* is one of the few organisms which can perform this work, provided that it is supplied with the means of obtaining the energy required for the process in the form of appropriate carbohydate nutrition. According to most authorities, the bacillus will do the same thing when cultivated outside the plant under strict experimental conditions.

After the bacilli have thriven for a while, mainly at the expense of the food supplied by the root in which they are living and multiplying, many of the individuals become weaker and undergo a sort of degeneration, while a few pass into a resting stage, in which they become highly resistant to adverse conditions of life. The leguminous plant, which previously gave out carbohydrate food to the bacillus, then begins to receive: it receives from the degenerated mass of bacilli the stores of nitrogenous matter they have accumulated, and this is a good return for the sugars which it had previously expended. The comparatively few surviving bacilli serve to infect the soil, as the roots gradually rot, and then they are enabled to attack the roots of new leguminous plants.

Higher Plants.—The phenomena of symbiosis between root and fungi occur also in many plants higher than the leguminous, for instance, in forest trees. In these trees the fungus mycelium forms a sort of mantle round the root, in contrast to being inside the cells of the cortex. The association is common in Castanea and Corylus, the rootlets so infected generally taking a characteristic coralloid appearance.

Importance of Symbiosis in the Phenomena of Reproduction.—Germination of Seeds: Noel Bernard, in 1902, proved experimentally that certain seeds, as the seeds of orchids, can germinate only in the presence of appropriate fungi. If the seedling is not infected with the appropriate fungus, generally the same fungus which infects the roots of the adult parent plant, germination, as a rule, will not take place, or it will be slow and imperfect. Burgeff came to the same conclusion: viz., that orchid seeds germinate without difficulty when the appropriate fungus is supplied to them. It is interesting to note that before this was scientifically known, orchid growers had found it necessary to sow orchid seeds in the soil containing the parent plant in order to make them grow; the explanation is that the seed in this way became infected with the fungi necessary to induce germination, viz., the fungus found in the roots of orchids.

A few more details may be of interest: Orchid seeds taken from the capsule under sterile conditions and sown on ordinary substrata where fungus is not present do not, as a rule, develop. The only phenomenon observed in these conditions is that usually the seeds swell, and after two or three months—viz., after the reserve food, oil, part of which becomes transformed into starch, has been exhausted—they become green and soon after die, unless the fungus infestation takes place. At first it seems peculiar that the seeds should die after the production of chlorophyle: one would rather expect that autonomous growth by action of photosynthesis should take place; as T. Ramsbottom aptly put it, it appears that the seedling forms chlorophyle as a sort of a last despairing effort.

Importance of Symbiosis in Fermentation Phenomena.—Two organisms, neither of which alone produces gas-fermentation in certain carbohydrates, may do so when living in symbiosis or artificially mixed together. This phenomenon is sometimes referred to as Castellani's phenomenon.

During the years 1904 and 1905, while in the East and in London on leave, I became interested in the study of baker's yeast and in fermentation caused by yeast. I made the following observations, mentioned at meetings of the Ceylon Branch of the British Medical Association.

1. Baker's yeast, both in the East and in London, is not a pure culture of a single species of yeast. It is generally composed of two or more species of yeast plus one or two or several species of bacteria. The bacteria isolated from samples of yeast in the East and in London do not produce gas-fermentation in sugars—they produce simple acidity.

2. Comparing the fermentation reactions of baker's yeast used in toto as obtained from the bakers, sowing the particles of the sample in the various sugars, with the fermentation reactions of the isolated pure cultures of the different species of saccharomyces and bacteria composing the sample, I found that the yeast in toto often ferments a larger number of carbohydrates than any one of the saccharomyces and bacteria that compose the sample.

3. By mixing artificially all the organisms isolated from the sample of yeast, in certain cases the same fermentation reactions are obtained as with the yeast in toto. There are apparent exceptions, as shown by the following example. From Orpington, a place in the country near London, I obtained in 1919 a sample of baker's yeast which gas-fermented a large number of carbon compounds, including starch. I investigated the sample mycologically by plating on maltose and dextrose agar, etc., and I isolated and grew in pure cultures all the organisms, saccharomyces and bacteria apparently present in that sample. By mixing all the pure cultures, I obtained the same fermentation reactions as with the sample of yeast in toto, except that the mixture did not ferment starch. The experiment was repeated many times with the same result, until finally I had recourse to the following procedure: the surface of the dextrose agar plates on which a minute particle of the yeast-cake had been plated out was washed with a little peptone water and the washing inoculated into starch. Fermentation took place, the inference being that probably some minute colonies on the verge of invisibility had escaped my notice when I investigated the plates and picked out all the colonies present; the thought came to me also that there might be an ultramicroscopic germ present.

4. By mixing artificially a nonmaltose fermenting saccharomyces (isolated from a sample of baker's yeast) with a bacillus isolated from the same sample, producing acidity in maltose and several other sugars, but never gas in any substance, I obtained fermentation of maltose with the production of gas.

5. In Ceylon by mixing two saccharomyces isolated locally, neither of which alone produced gas or acidity in maltose, gas fermentation of that sugar was obtained.

In recent years I have studied the same and similar phenomena, using mostly pathogenic bacteria: bacilli and cocci, also a pathogenic yeast.

"*Symbiotic Fermentation Phenomenon.*"—As I have already stated, the phenomenon I have described may be defined as follows: two organisms, neither of which alone produces gas fermentation, may do so when mixed together; for instance, *Cryptococcus graciloides* alone will not produce gas in dextrose; *B. vermiculoides* alone will not produce gas in that sugar; when the two organisms live in symbiosis or are artificially mixed they will produce gas. This phenomenon is evident when certain bacteria are used; for instance, *B. typhosus* alone does not produce any gas in maltose (acid only); *B. morgani* alone does not produce any gas in that sugar (neither acidity nor gas): the mixture *B. typhosus* + *B. morgani* produces gas.

Use of the Symbiotic Fermentation Phenomenon in the Differentiation of Certain Micro-Organisms.—The symbiotic fermentation phenomenon may be used to differentiate certain micro-organisms; for instance, organisms of the dysentary group. The two principal types of dysentery organisms, Shiga and Flexner *sensu lato*, may be differentiated by the use of their symbiotic fermentation with *Bacillus morgani.* Shiga + Morgan will not produce gas in mannitol; Flexner + Morgan will produce gas. It may be said generally that the results of the symbiotic fermentation largely correspond to the results obtainable with simple acid fermentation, viz., there will be gas in those carbohydrates in which the organism to be identified will produce, alone, simple acidity; the presence of gas, however, is much more striking than simple acid fermentation; it may, moreover, in certain cases become rapidly evident, while the simple production of acid may be slow. For instance, there has been some difference of opinion on the point as to whether *Bacillus dysenteriae* Shiga-Kruse produces acidity in maltose. In old textbooks on bacteriology the organism is described as producing slight acidity in maltose; in most of the modern books it is described as not producing acidity. The symbiosis Shiga + Morgan produces gas in maltose, and this favors the possibility of Shiga producing a certain amount, however small, of acidity in that sugar.

Differentiation of Bacillus dysenteriae Shiga-Kruse, Bacillus dysenteriae Flexner, Bacillus dysenteriae Y (Hiss-Russell), by Means of Symbiotic Fermentation in Mannitol and Maltose.—The symbiosis Shiga + Morgan produces gas in maltose, not in mannitol; the symbiosis Flexner + Morgan produces gas in maltose and mannitol; the symbiosis Y + Morgan produces gas in mannitol, not in maltose.

Identification of Certain Carbon Compounds by Means of the Symbiotic Fermentation Phenomenon.—The phenomenon is useful in the identification and differentiation of certain carbon compounds. For instance, a Fehling-reducing substance which is gas-fermented by *Bacillus typhosus* + *Bacillus morgani*, and not by *Bacillus kandiensis* + *Bacillus morgani*, is probably maltose. The explanation is as follows: The symbiosis *Bacillus typhosus* + *Bacillus morgani* and the symbiosis *Bacillus kandiensis* + *Bacillus morgani* produce gas in the same Fehling-reducing sugars except maltose, in which *Bacillus typhosus* + *Bacillus morgani* produces gas and *Bacillus kandiensis* + *Bacillus morgani* does not. If a Fehling-reducing sugar, therefore, is gas-fermented by *Bacillus typhosus* + *Bacillus morgani* and not by *Bacillus kandiensis* + *Bacillus morgani* the inference is that it is maltose. For the identification of maltose and other carbon compounds, however, the mycologic method I devised in Ceylon and later worked out in England with Dr. F. E. Taylor is much simpler, as will be shown later.

Importance of Symbiosis in the Etiology of Certain Diseases and in the Causation of Certain Symptoms.—There are certain diseases which are, in all probability, caused by the symbiosis of two vegetal organisms such as a fungus and a bacterium; for instance, *Trichomycosis nigra* and *Trichomycosis rubra*.[14]

MYCOLOGIC METHOD TO DETECT AND IDENTIFY VARIOUS CARBOHYDRATES

Some years ago while I was in Ceylon I devised a mycologic method for the detection and identification of various carbohydrates. This method I worked out later on in London in the Kings College Laboratories with Dr. F. E. Taylor. A joint paper was published in the *British Medical Journal*[15] Recently, we have published again a fairly complete description of the method in *The Journal of the American Medical Association*.[16] We do not have specific germs for each substance, germs, for instance, that will ferment only maltose, only galactose or only inulin, but as a number of fungi and bacteria select certain carbohydrates to the exclusion of others I found it possible, by using certain species and comparing the results, to devise a general method. To understand the working of the method it is necessary to keep in mind the biochemical fermentative characters of certain fungi and bacteria, which are collected in table 11. I will limit myself to a brief description of the identification and detection of maltose, galactose and inulin, and I will also give a list of the principal mycologic formulas we have devised and employed in the identification of various carbon compounds.

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[14] Castellani, Aldo: Fungi and Fungous Diseases; Lecture III, Arch. Dermat. & Syph., to be published.

[15] Castellani, Aldo, and Taylor, F. E.: Brit. M. J. **2**:855 (Dec. 29) 1917.

[16] Castellani, Aldo, and Taylor, F. E.: Fallacies of "Yeast Method" of Detecting Glucose in Urine, Further Observations on Mycologic Method to Identify Various Sugars and Other Carbon Compounds, J. A. M. A. **86**:523 (Feb. 20) 1926.

Identification of Maltose.—In order to determine whether a given substance is maltose or not, a sterile 1 per cent solution in sugar-free peptone water is made and distributed into two tubes, each containing a Durham's fermentation tube or similar appliance. One of the following procedures is then used:

A. A 1 per cent sterile solution in sugar-free peptone water is made of the substance to be tested for maltose. The solution is distributed into two tubes, which are labeled 1 and 2. Tube 1 is inoculated with *Monilia tropicalis* and tube 2 with *Monilia macedoniensis*, or other monilias of the same group (*M. macedoniensoides*). If, after four days' incubation at 37 C., tube 1 contains gas and tube 2 does not, it can be concluded that the substance is maltose. This is easily understood if one keeps in mind the biochemical reactions of the two monilias. *M. tropicalis* ferments, with the production of gas, the following substances: dextrose, levulose, galactose, maltose and saccharose. *M. macedoniensis* and all other monilias of the macedoniensis group (*M. macedoniensoides*, etc.) ferment, with the production of gas, the following carbon compounds: dextrose, levulose, galactose, saccharose and inulin.

The substance being investigated has been fermented with the production of gas by *M. tropicalis*. It can be, therefore, only dextrose, levulose, maltose, galactose or saccharose, but, if it had been dextrose, levulose, galactose or saccharose, it would have been fermented with production of gas also by *M. macedoniensis*. Therefore, it can only be maltose.

$$\left.\begin{array}{ll}\textit{Monilia tropicalis}\ \text{Castellani} & + \\ \textit{Monilia macedoniensis}\ \text{Castellani} & 0\end{array}\right\} = \text{maltose}$$

B. Tube 1 is inoculated with *Monilia pinoyi* and tube 2 with *Monilia krusei*. The two tubes are placed in an incubator at from 35 to 37 C. for three days. If, after that time, tube 1 (*Monilia pinoyi*) contains gas and tube 2 (*Monilia krusei*) does not, it can be concluded that the substance is maltose. This is easily understood if one keeps in mind the fermentative reactions of the two monilias. *Monilia pinoyi* ferments with the production of gas only the following carbon compounds: dextrose, levulose and maltose. *Monilia krusei* ferments, with the production of gas, only dextrose and levulose.

The substance under investigation has been fermented, with the production of gas, by *Monilia pinoyi*; it can be only dextrose, levulose or maltose, but if it had been either dextrose or levulose, it would have been fermented also by *Monilia krusei*; therefore, it can be only maltose.

$$\left.\begin{array}{ll}\textit{Monilia pinoyi} & + \\ \textit{Monilia krusei} & 0\end{array}\right\} = \text{maltose}$$

C. Tube 1 is inoculated with *M. metalondinensis* and tube 2 with *M. macedoniensis* or *M. macedoniensoides*. The two tubes are incubated at 37 C. for four days. If, after that time tube 1 contains gas and tube 2 does not, the substance is maltose. This is explained by the fact that *M. metalondinensis* ferments with the production of gas only dextrose, levulose, maltose and galactose; and *M. mace-*

doniensis only the following carbon compounds: dextrose, levulose, galactose, saccharose and inulin.

The substance now being investigated has been fermented by *M. metalondinensis.* It can be, therefore, only dextrose, levulose, maltose or galactose; but, if it were dextrose, levulose or galactose, it would have been fermented also by *M. macedoniensis.* There is only one possibility left—it must be maltose.

$$\left.\begin{array}{ll}\textit{Monilia metalondinensis}\text{ Castellani} & + \\ \textit{Monilia macedoniensis}\text{ Castellani} & 0\end{array}\right\} = \text{maltose}$$

Identification of Maltose when Present with Other Common Fermentable Substances (Dextrose, Levulose, Galactose, Saccharose).—If a liquid is suspected of containing maltose mixed with dextrose, levulose, galactose and saccharose, it is fermented with *Monilia macedoniensis* or *M. macedoniensoides.* If after exhaustion with *M. macedoniensis,* the liquid is still fermented, with the production of gas, by *Monilia pinoyi, Monilia tropicalis* or *Monilia metalondinensis,* the conclusion is that the liquid also contains maltose. Strains of the various monilias should be used that have approximately the same fermentative power on the various carbohydrates they all ferment.

Identification of Galactose.—In determining whether or not a certain substance is galactose, the following procedures are used:

A. A 1 per cent sterile solution of the substance is made in sugar-free peptone water and distributed into two tubes, each containing a fermentation tube. Tube 1 is inoculated with *M. metalondinensis* and tube 2 with *M. pinoyi.* If, after four days' incubation at 37 C., tube 1 contains gas, and tube 2 does not, the substance must be galactose. The explanation lies in the fact that *M. metalondinensis* ferments only the following carbon compounds: dextrose, levulose, maltose and galactose; *M. pinoyi* ferments only the following substances: dextrose, levulose and maltose.

The substance has been fermented by *M. metalondinensis.* It can be, therefore, only dextrose, levulose, maltose or galactose. It has not been fermented by *M. pinoyi,* so it cannot be dextrose, levulose or maltose. There is only one possibility left—it must be galactose.

$$\left.\begin{array}{ll}\textit{Monilia metalondinensis} & + \\ \textit{Monilia pinoyi} & 0\end{array}\right\} = \text{galactose}$$

B. The solution is distributed into three tubes, each containing a fermentation tube. Tube 1 is inoculated with *M. metalondinensis,* tube 2 with *M. krusei* and tube 3 with *M. macedoniensis.* The three tubes are placed in an incubator at 37 C. for four days. If, after that period of time, tubes 1 and 3 contain gas and tube 2 does not, the substance is galactose.

This is easily explained if one keeps in mind the biochemical reactions of the three fungi. *M. metalondinensis* produces gas only in dextrose, levulose, maltose and galactose; *M. macedoniensis* produces gas only in dextrose, levulose, galactose, saccharose and inulin; *M. krusei* produces gas only in dextrose and levulose.

The substance now under investigation has been fermented, with the production of gas, by *M. metalondinensis.* It can be, therefore, only dextrose, levulose, maltose or galactose. It cannot be dextrose or levulose, because in such a case it

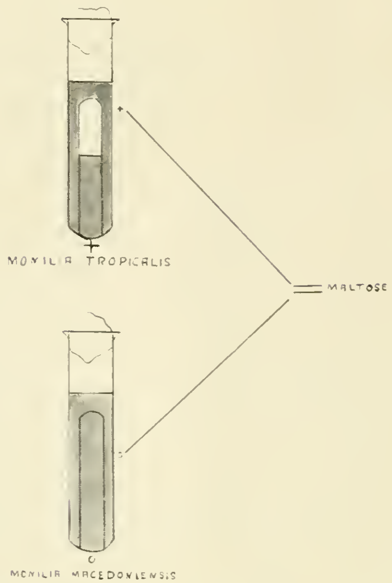

Fig. 44.—Identification of maltose.

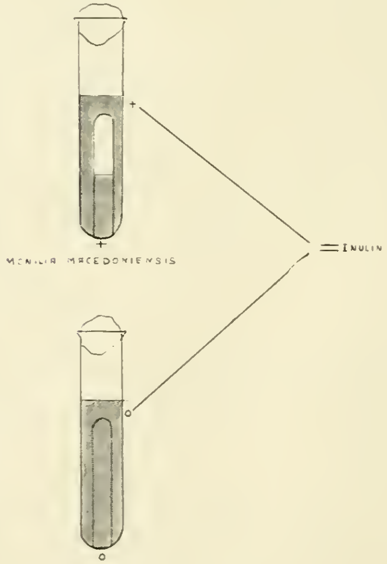

Fig. 45.—Identification of inulin.

would have been fermented by *M. krusei*, and it cannot be maltose, because if it were it would not have been fermented by *M. macedoniensis*. There is only one possibility left—it must be galactose.

Monilia metalondinensis	+	= galactose
Monilia krusei	0	
Monilia macedoniensis	+	

C. The solution is distributed into three tubes: Tube 1 is inoculated with *M. macedoniensis*, tube 2 with *M. krusei* and tube 3 with *B. paratyphosus* B. If, after four days in the incubator at 37 C., tube 1 shows presence of gas, tube 2 shows absence of gas and tube 3 shows presence of gas, it can be concluded that the substance under investigation is galactose. As the substance has been gas-fermented by *M. macedoniensis* it can be only dextrose, levulose, galactose, saccharose or inulin; but it has not been fermented by *Monilia krusei*, therefore it cannot be dextrose or levulose; it can only be galactose, saccharose or inulin, but had it been saccharose or inulin it would not have been fermented by *B. paratyphosus* B. There is only one possibility left—it must be galactose.

Monilia macedoniensis Castellani	+	= galactose
Monilia krusei Castellani	0	
Bacillus paratyphosus B	+	

D. The solution is distributed into two tubes; tube 1 is inoculated with *B. morgani* (strain S) and tube 2 with *Monilia krusei*. If, after three days in the incubator at 35 C., tube 1 contains gas and tube 2 does not, it can be concluded that the substance is galactose, provided the substance is known to be a carbohydrate.

This is easily understood by keeping in mind the fermentation reactions of the two germs. *Bacillus morgani* (strain S) ferments, with production of gas, glucose, levulose and galactose. The substance has been gas-fermented by *B. morgani;* if it is a carbohydrate it can only be dextrose, levulose or galactose. It has not been fermented by *Monilia krusei;* it cannot, therefore, be dextrose or levulose; it must be galactose.

B. morgani Castellani and Chalmers	+	= galactose
Monilia krusei Castellani	0	

Identification of Galactose When Present with Other Common Fermentable Substances (Dextrose, Levulose, Maltose, Saccharose).—To detect galactose in a liquid in which it is suspected to be present together with dextrose, levulose, maltose or saccharose, the liquid is inoculated with an organism which gas-ferments all the sugars I have mentioned except galactose; for instance, *Monilia bronchialis*. If, after exhaustion with *M. bronchialis* the liquid is still fermentable with *Monilia tropicalis*, the inference is that the liquid contained galactose, in addition to other fermentable substances.

USE OF MYCOLOGIC METHOD IN URINANALYSIS

1. The urine must be aseptic; if it cannot be collected aseptically, it should be sterilized as soon as possible after distribution in the tubes, by heating in Koch's steamer for thirty minutes. Once is sufficient for practical purposes, but in special cases, it may be heated on two or three

consecutive days. It should never be autoclaved, as this procedure may alter the character of the sugars and other carbohydrates present. Another procedure is to filter the sample of urine by means of a Chamberland or Berkefeld filter. I have recently found that boiling the urine for a few seconds does not apparently alter the chemical structure of the various sugars, and in practice this procedure is much simpler.

Table 13.—*Identification of Certain Fehling-Reducing Substances* *

Monilia balcanica Castellani	Monilia krusei Castellani	Monilia pinoyi Castellani	Monilia metalondinensis Castellani	Bacillus paratyphosis B Schottmüller	Bacillus coli Escherich	Reducing Substances
+	+	+	+	+	+	Dextrose
0	+	+	+	+	+	Levulose
0	0	+	+	+	+	Maltose
0	0	0	+	+	+	Galactose
0	0	0	0	+	+	Pentose
0	0	0	0	0	+	Lactose
0	0	0	0	0	0	Nonfermentable substances of the groups creatinine, hippuric acid, uric acid, etc.

* The reducing substance is made into a 1 per cent peptone water solution. Selected strains of the various organisms with permanent biochemical reactions and which produce a large amount of gas should be used.

Table 14.—*Key to Identification of Certain Fehling-Reducing Substances*

Inoculate tube 1 with Monilia balcanica Castellani	+ = Dextrose					
	If negative inoculate tube 2 with M. krusei Castellani	+ = Levulose				
		If negative inoculate tube 3 with M. pinoyi Castellani	+ = Maltose			
			If negative inoculate tube 4 with M. metalondinensis Castellani	+ = Galactose		
				If negative inoculate tube 5 with B. coli Escherich and tube 6 with B. paratyphosus B Schottmüller	B. coli 0 B. paratyphosus 0	Nonfermentable reducing substances of the groups creatinine, uric acid, hippuric acid, etc.
					B. coli + B. paratyphosus B 0	= Lactose
					B. coli + B. paratyphosus B +	= Pentose

2. It is of great advantage to add a third or the same amount of peptone water to the urine; otherwise, the organisms may, at times, grow scantily, and there may not be a distinct production of gas.

If a urine is Fehling-reducing, one can determine which of the six sugars found in pathologic urines (dextrose, levulose, maltose, galactose, lactose, pentose) is present, by the following procedure:

The urine is distributed into six tubes, each containing a Durham's fermentation tube, or saccharimeters may be used. They are labeled 1, 2, 3, 4, 5 and 6. Tube 1 should be inoculated with *Monilia balcanica* Castellani, or *M. parabalcanica* Castellani; tube 2 with *M. krusei* Castellani; tube 3 with *M. pinoyi* Castellani; tube 4 with *M. metalondinensis* Castellani; tube 5 with *B. coli-communior* Escherich, and tube 6 with *B. paratyphosus* B Schottmüller.

After the tubes are incubated at 35 C. for four days, the results may be read:

1. If in tubes 1, 2, 3, 4, 5 and 6 gas is present, the reducing substance is dextrose.

2. If tube 1 does not contain any gas, and in tubes 2, 3, 4, 5 and 6 gas is present, the reducing substance is levulose.

3. If tubes 1 and 2 do not contain gas, and in tubes 3, 4, 5 and 6 gas is present, the reducing substance is maltose.

4. If tubes 1, 2 and 3 do not contain gas, and in tubes 4, 5 and 6 gas is present, the reducing substance is galactose.

5. If tubes 1, 2, 3 and 4 do not contain gas, and in tubes 5 and 6 gas is present, the reducing substance is pentose.

6. If tubes 1, 2, 3, 4 and 6 do not contain gas, and in tube 5 gas is present, the reducing substance is lactose.

7. If none of the tubes contains gas, the reducing substance belongs to the nonfermentable group of reducing substances, including uric acid, creatinine, hippuric acid and others.

PRESENCE IN THE URINE OF MORE THAN ONE REDUCING SUBSTANCE

If the presence of more than one sugar and other carbohydrates is suspected, this may, to a certain extent, be ascertained and the various carbohydrates determined by the mycologic method. If it is assumed that the urine, after gas fermentation with *M. balcanica* Castellani, is still fermentable with production of gas by *M. krusei* Castellani, the conclusion is that in addition to dextrose the urine must have contained levulose; strains of *M. balcanica* Castellani, and *M. krusei* Castellani, having approximately the same fermentation power on dextrose should be used. If, then, after exhaustion, first with *M. balcanica* Castellani, and then with *M. krusei* Castellani, the urine is fermentable with production of gas by *B. morgani* Castellani and Chalmers the presumption is that it contained three sugars—dextrose, levulose and galactose. The explanation is found in the fact that *M. balcanica* Castellani ferments, with the production of gas, glucose only; *M. krusei* Castellani, dextrose and levulose; *B. morgani* Castellani and Chalmers, dextrose, levulose and galactose. If a Fehling reducing urine after exhaustion with *Monilia tropicalis* is capable of being fermented by *B. coli* and not by *B. paratyphosus* B, or is capable of being fermented by *Monilia pseudotropicalis*, it means that the urine in addition to dextrose, levulose, maltose or galactose, or a mixture of those sugars, contained also lactose.

Maltosuria.—Maltose, as is well known, reduces Fehling, and cases of maltosuria have naturally been mistaken for true glycosuria. For the detection of maltose, the urine is obtained aseptically by catheter, or if this is not feasible it is collected in a sterile receptacle and distributed at once in sterile tubes, each containing a small fermentation tube. The tubes are sterilized in Koch's stove for thirty minutes once or twice at an interval of six hours. This, in my experience, was generally sufficient, but at times it is advisable to repeat the sterilization in Koch's stove on two or three consecutive days. The urine should never be autoclaved, as this procedure may alter the composition of the sugars and other carbohydrates present. The urine freshly passed may also be filtered through a Chamberland filter instead of being sterilized. The tubes of aseptic urine are inoculated with the monilias, as explained previously. If maltose is suspected to be present in conjunction with other fermentable substances, such as dextrose, levulose and galactose, the urine is exhausted with *Monilia macedoniensis.* If, after exhaustion with *Monilia macedoniensis,* the urine is still fermentable when inoculated with *Monilia tropicalis, Monila metalondinensis* or *Monilia pinoy,* it can be concluded that the urine contains maltose in addition to other fermentable substances.

REPORT OF ILLUSTRATIVE CASE

An Englishman, aged 47, a tea planter, had been a heavy beer drinker for many years. He was feeling quite well, except that his stoutness made it difficult for him to go for long walks, and this interfered with his duties. A substance that reduced Fehling's solution was found in his urine several times during the previous glycosuria which, it was thought, might develop into true diabetes. He came to me for consultation. The urine reduced Fehling's solution and was investigated for the presence of the following carbohydrates which reduce Fehling's solution: dextrose, levulose, galactose, maltose, lactose and pentose. The patient passed urine in my presence, into two sterile vessels; the urine of the second vessel was distributed immediately by means of a sterile pipet into five tubes of peptone water (two parts of urine to one of peptone water), each containing a fermentation tube. The tubes were labelled numbers 1 to 5. Number 1 was inoculated with *Monilia krusei* Castellani, number 2 with *Monilia pinoyi* Castellani, number 3 with *Monilia metalondinensis* Castellani, number 4 with *Bacillus coli* Escherich and number 5 with *Bacillus paratyphosus* B Schottmüller. The five tubes were placed in the incubator at 37 C. for four days. On examination the results were as follows:

Tube 1 *(Monilia krusei)*	0
Tube 2 *(Monilia pinoyi)*	+
Tube 3 *(Monilia metalondinensis)*	+
Tube 4 *(Bacillus coli)*	+
Tube 5 *(Bacillus paratyphosus* B)	+

This result according to my method means presence of maltose. The urine was gas-fermented by *Monilia pinoyi* and not by *Monilia krusei. Monilia pinoyi* produces gas only in three carbon compounds, viz., dextrose, levulose and maltose. the urine, therefore, must contain dextrose, levulose or maltose, or a mixture of these various sugars, but if the urine contained either dextrose or levulose it would have been gas-fermented also by *Monilia krusei.* The result obtained by my mycologic method was confirmed by the carrying out of purely chemical methods by a well known chemist.

Lactosuria.—Next to dextrose, lactose is the commonest sugar found in the urine. It is encountered frequently in the urine of nursing mothers. Lactose, as is well known, reduces Fehling's solution, and not infrequently lactosuria has been mistaken for true glycosuria. It is most important to distinguish between the two conditions, as lactosuria is not serious, while glycosuria, especially in nursing women, is of grave prognosis. The chemical detection and determination of lactose is difficult for an ordinary medical man. In most textbooks it is stated that if a urine reduces Fehling's solution, and is not fermented by ordinary baker's yeast (so-called German yeast, or baker's yeast), the presumption is that the reducing substance is lactose. There are, however, two important sources of error.

First, a number of specimens of baker's yeast ferment lactose in addition to dextrose, maltose and other sugars; second, even if the baker's yeast should not ferment lactose, this is not the only substance that reduces Fehling's solution which may not be fermented by baker's yeast—pentose, for instance.

If a urine which reduces Fehling's solution is not fermented by ordinary yeast, it cannot, therefore, be concluded that the condition is lactosuria.

Detection and Determination of Lactose by the Mycologic Method.—This method can be carried out in various ways. A few mycologic formulas are cited as examples.

In a urine that reduces Fehling's solution, for practical purposes, the following formulas are correct:

I. *Bacillus coli* Escherich	+	= lactose
Bacillus paratyphosus B Schottmüller	0	

Bacillus coli and *Bacillus paratyphosus* B, besides lactose, differ in their action on raffinose and salicine and glycerol, but raffinose and salicine are not carbon compounds found in the urine and at any rate they do not reduce Fehling's solution. The same formula applies to raffinose, salicine and glycerol, but there are substances which are of rare occurrence in the urine and, moreover, do not reduce Fehling's solution; therefore, this formula may be considered to be specific for practical purposes.

II. *Bacillus neapolitanus* Emmerich	+	= lactose
Bacillus pseudoasiaticus Castellani	0	

The explanation lies in the fact that *Bacillus neapolitanus* and *Bacillus pseudoasiaticus* differ only in lactose as regards sugars and other carbohydrates found in pathologic urines.

III. *Monilia metalondinensis or Monilia tropicalis* Castellani	0	= lactose (with great probability)
Bacillus coli Escherich	+	
Bacillus paratyphosus B Schottmüller	0	

IV. *Monilia pseudotropicalis* Castellani	+	= lactose
Monilia macedoniensis Castellani	0	

V. *Monilia pseudotropicalis* Castellani	+	= lactose
Monilia tropicalis Castellani	0	

The technical details for carrying out the test are identical with those already mentioned in connection with the search for maltose, etc.

Pentosuria.—Pentosuria is not a rare condition. As is well known, the pentoses reduce Fehling's solution well and Nylander solution little; they give a positive reaction with Bial's and Read's modifications of the orcein test.

The mycologic method can be used for the detection of pentose in a urine that reduces Fehling's solution according to the following formulas:

Monilia tropicalis	0	= pentose (generally arabinose)
Bacillus coli Escherich	+	
Bacillus paratyphosus B	+	

Monilia metalondinensis	0	= pentose (generally arabinose)
Bacillus coli Escherich	+	
Bacillus paratyphosus B	+	

REPORT OF ILLUSTRATIVE CASE

Some time ago I was consulted in London by a planter, aged 38, who had been sent home from a tropical country in the Far East, with his condition diagnosed as diabetes. His general condition, however, was fairly good, and he said that he had not lost much weight. According to his statement he was not thirsty or hungry, nor did he feel weak. He appeared somewhat nervous and irritable, however, and was worried at the idea of suffering from diabetes. The urine passed during twenty-four hours varied between 3 and 4 pints. It was slightly acid, with a density of 1.020. It persistently reduced Fehling's solution well, and apparently the diagnosis of diabetes had been based on this fact.

The urine was examined by the mycologic method for the presence of the following carbohydrates which reduce Fehling's solution: dextrose, levulose, galactose, maltose, pentose and lactose. The patient was made to pass urine into three sterile vessels; the urine of the second vessel was distributed immediately by means of a sterile pipet into six tubes of peptone water (about two parts of urine to one of peptone water), each containing a fermentation tube. The tubes were labelled numbers 1 to 5. Tube 1 was inoculated with *Monilia krusei* Castellani; tube 2 with *Monilia pinoyi* Castellani; tube 3 with *Monilia metalondinensis* Castellani; tube 4 wth *Bacillus coli* Escherich; tube 5 with *Bacillus paratyphosus* B Schottmüller. The five tubes were placed in the incubator at 37 C. and examined after forty-eight hours. The following were the results:

Tube 1 (*Monilia krusei* Castellani)................	0
Tube 2 (*Monilia pinoyi* Castellani).................	0
Tube 3 (*Monilia metalondinensis* Castellani).......	0
Tube 4 (*Bacillus coli* Escherich)..................	+
Tube 5 (*Bacillus paratyphosus* B Schottmüller)....	+

According to my method, these results in a urine that reduces Fehling's solution mean the presence of pentoses.

The result obtained by the mycologic test was confirmed by chemical investigation. The urine which reduced Fehling's solution well and Nylander solution little gave a positive reaction for pentoses with Bial's and Read's modifications of the orcein test. There can be little doubt, therefore, that the patient was suffering from "pentosuria" and not from diabetes. It is important in practice to try to differentiate in every case betwen glycosuria and pentosuria, as the first condition is often of grave prognosis, while the second points to a much more benign disturbance of metabolism and practically does not require any treatment.

Saccharosuria.—Spontaneous saccharosuria is an extremely rare condition, but artificial saccharosuria is not so rare among malingerers. During the Great War I encountered three cases in which saccharose had been added to the urine with the object of misleading the medical officer. The physician who uses as routine the usual Fehling method is not apt to diagnose such cases as glycosuria, as saccharose does not reduce Fehling's solution—at any rate not clearly; but the medical man who uses what is considered by most authorities the more exact method, that of yeast fermentation, will likely fall into error, because though in so many textbooks it is stated that dextrose is the only substance fermented by ordinary baker's yeast, I have found that a large number of specimens of baker's yeast will ferment saccharose and also levulose, maltose, etc., in addition to dextrose.

Mycologic Test to Determine Saccharose.—According to my method, various tests may be evolved to determine whether a certain carbohydrate of those found in pathologic urine is or is not saccharose. A few of these tests are presented here in the convenient shape of mycologic formulas:

$$\text{I. }\left.\begin{array}{r}\textit{Monilia metalondinensis}\text{ Castellani}\ldots\ldots 0\\ \textit{Monilia tropicalis}\text{ Castellani}\ldots\ldots\ldots\ldots +\end{array}\right\} = \text{saccharose}$$

This is explained by the fact that *Monilia metalondinensis* and *Monilia tropicalis* are identical in all their biochemical reactions except on saccharose, which is fermented with production of gas by *Monilia tropicalis* and not fermented by *Monilia metalondinensis.*

$$\text{II. }\left.\begin{array}{r}\textit{Monilia pinoyi}\text{ Castellani}\ldots\ldots\ldots\ldots\ldots 0\\ \textit{Monilia rhoi}\text{ Castellani}\ldots\ldots\ldots\ldots\ldots\ldots +\end{array}\right\} = \text{saccharose}$$

The explanation lies in the fact that *Monilia pinoyi* ferments, with production of gas, only dextrose, levulose and maltose, and *Monilia rhoi* only dextrose, levulose, maltose and saccharose. If a substance, therefore, is not fermented by *Monilia pinoyi* and is fermented, with production of gas, by *Monilia rhoi*, it must be saccharose.

To the foregoing purely mycologic formulas is added a chemicomycologic formula which may be found useful in practice:

$$\left.\begin{array}{r}\text{Fehling}\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 0\\ \textit{Monilia tropicalis}\text{ Castellani}\ldots +\end{array}\right\} = \text{saccharose}$$

This is explained by the fact that *Monilia tropicalis* ferments with production of gas, dextrose, levulose, maltose, galactose, saccharose and no other substance. If Fehling's solution gives a negative reaction, it cannot be any of the first four substances that is present, as they all reduce Fehling's solution; it must, therefore, be saccharose.

Detection of Saccharose when Mixed with Other Fermentable Substances.—The urine should be exhausted with *Bacillus coli* Escherich *sensu stricto* (does not ferment saccharose), and later, after filtration through a Chamberland filter, or prolonged centrifugalization, it should be inoculated with *Monilia tropicalis.* If the urine is still fermentable, the presumption is that in addition to other fermentable substances which can be found by the general method, the urine contains saccharose.

Inosituria.—Inosite, as is well known, does not reduce Fehling's solution; it is not really a sugar, and belongs to the aromatic series. It is not of rare occurence in the urine, and is, for instance, not infrequently found in diabetes insipidus, but the chemical methods of detecting it are far from easy or rapid. On the other hand, inosite, provided it is present in the urine in not too small a quantity, can be detected and determined with comparative ease by the mycologic method.

Two tubes of urine collected aseptically, or sterilized as already described, are mixed with one-third, or the same amount, of sugar-free peptone water and inoculated: number 1 with *Bacillus paratyphosus* B Schottmüller (M variety, which ferments inosite), number 2 with *Bacillus paratyphosus* A Schottmüller (does not ferment inosite). The two germs, as regards carbohydrates and other pathologic substances found in the urine, differ only in their action on inosite. Therefore, if, after forty-eight hours' incubation at 37 C., gas is present in tube 1, and absent in tube 2, one has to deal with inosite. This can be expressed conveniently by the following formula:

Bacillus paratyphosus B Schottmüller, var. M +
Bacillus paratyphosus A Schottmüller......... 0
= inosite

If the result of the test is

Bacillus paratyphosus B Schottmüller, var. M........ 0
Bacillus paratyphosus A Schottmüller............... 0

it means that the substance is neither inosite nor any other substance fermentable by *Bacillus paratyphosus* B, var. M, or *Bacillus paratyphosus* A; hence, it cannot be dextrose, levulose, maltose, saccharose, etc.

If the result of the test is

Bacillus paratyphosus B Schottmüller, var. M.........+
Bacillus paratyphosus A Schottmüller................+

the urine should be exhausted with *Bacillus paratyphosus* A, and then inoculated with *Bacillus paratyphosus* B, var. M, using strains having approximately the same fermentation power. If gas is produced, the inference is that the urine contained inosite in addition to other fermentable substances, which can be determined by using the general mycologic method.

SUMMARY OF THE GENERAL MYCOLOGIC METHOD

The entire subject can be condensed into the following formulas:

Mycologic Formulas

DEXTROSE

Monilia balcanica Castellani...........+ = dextrose

LEVULOSE

Monilia krusei Castellani..............+
Monilia balcanica Castellani........... 0
= levulose

MALTOSE

Monilia tropicalis Castellani...........+
Monilia macedoniensis (*Monilia macedoniensoides*) Castellani............. 0
= maltose

Monilia metalondinensis Castellani.....+
Monilia macedoniensis Castellani, or
Monilia macedoniensoides 0
= maltose

Monilia pinoyi Castellani ....+
Monilia krusei Castellani............. 0
= maltose

Monilia pinoyi Castellani...............+
Monilia macedoniensis (*Monilia macedoniensoides*) Castellani........... 0
= maltose

Monilia tropicalis Castellani...........+
Monilia rhoi Castellani................ 0
= maltose

Monilia pinoyi Castellani	+	= maltose
Bacillus morgani Castellani and Chalmers	0	

Monilia metalondinensis Castellani	+	= maltose
Bacillus morgani Castellani and Chalmers	0	

GALACTOSE

Monilia metalondinensis Castellani	+	= galactose
Monilia pinoyi Castellani	0	

Monilia metalondinensis Castellani	+	= galactose
Monilia kruseí Castellani	0	
Monilia macedoniensis Castellani	+	

Monilia tropicalis Castellani	+	= galactose
Monilia bronchialis Castellani	0	

Monilia macedoniensis Castellani	+	= galactose
Monilia krusei Castellani	0	
Bacillus paratyphosus B Schottmüller	+	

Bacillus morgani Castellani and Chalmers	+	= galactose
Monilia krusei Castellani	0	

SACCHAROSE

Monilia tropicalis Castellani	+	= saccharose
Monilia metalondinensis Castellani	0	

Monilia tropicalis Castellani	+	= saccharose
Bacillus coli-communis, sensu stricto (does not ferment saccharose) Escherich	0	

Monilia tropicalis Castellani	+	= saccharose
Bacillus paratyphosus B Schottmüller	0	

Monilia macedoniensis Castellani	+	= saccharose
Bacillus coli communis, sensu stricto Escherich	0	
Bacillus coli communior	+	

Monilia macedoniensis Castellani	+	= saccharose
Bacillus paratyphosus B Schottmüller	0	
Bacillus coli-communior	+	

Bacillus coli-communis, sensu stricto Escherich	0	= saccharose (with great probability)
Bacillus neapolitanus Emmerich	+	

Bacillus coli-communis, sensu stricto Escherich	0	= saccharose (with great probability)
Bacillus asiaticus Castellani	+	

INULIN

Monilia macedoniensis or *Monilia macedoniensoides* Castellani	+	= inulin
Monilia tropicalis Castellani	0	

Monilia macedoniensis Castellani *(Monilia macedoniensoides)*	+	= inulin
Monilia rhoi Castellani	0	

Monilia macedoniensis (Monilia macedoniensoides) Castellani	+	= inulin
Bacillus coli-communior Escherich; *Bacillus pseudocoli* Castellani; *Bacillus neapolitanus* Emmerich	0	

Monilia macedoniensis (Monilia macedoniensoides Castellani	+	= inulin
Bacillus asiaticus Castellani	0	

Monilia macedoniensis (or *Monilia macedoniensoides*	+	= inulin
Bacillus orleanensis	0	

INOSITOL

Bacillus paratyphosus B, strain M Schottmüller	+	= inositol (with great probability)
Bacillus paratyphosus A Schottmüller	0	

GLYCEROL

Bacillus asiaticus Castellani	+	= glycerol (with great probability)
Bacillus paratyphosus B, var. M Schottmüller	0	
Bacillus colombensis, strain L Castellani	+	

CHEMICOMYCOLOGIC FORMULAS

SACCHAROSE

Fehling's solution	0	= saccharose
Monilia tropicalis Castellani	+	

LACTOSE

Fehling's solution	+	= lactose
Monilia pseudotropicalis	+	
Monilia macedoniensis	0	

Fehling's solution	+	= lactose
Bacillus paratyphosus B Schottmüller	0	
Bacillus coli-communis (or *communior)* Escherich	+	

Fehling's solution	+	= lactose
Bacillus orleanensis	+	
Monilia macedoniensis	0	
Bacillus coli	+	

PENTOSE

Fehling's solution	+	= pentose (generally arabinose)
Monilia tropicalis Castellani	0	
Bacillus paratyphosus B Schottmüller	+	
Bacillus coli-communis Escherich	+	

As is the case with every other biologic method the results must be considered to be relatively and not absolutely certain.

ABRIDGED METHOD

The method I now use as a matter of routine is carried out using the following organisms:

1. *Monilia krusei* Castellani, 1909.
2. *Monilia tropicalis* Castellani, 1909.
3. *Monilia metalondinensis* Castellani.
4. *Monilia pinoyi* Castellani.
5. *Monilia pseudotropicalis* Castellani (strain IV).
6. *Monilia macedoniensis* Castellani, 1917 (var. *macedoniensoides).*
7. *Bacillus morgani* Castellani and Chalmers, 1918.
8. *Bacillus coli-communis* Escherich, 1886, *sensu stricto* (does not ferment saccharose).
9. *Bacillus coli-communior* Escherich-Durham, 1899 (var. *pseudocoscoroba).*
10. *Bacillus paratyphosus* B Schottmüller, 1902.

With the abridged method the following carbohydrates may be easily detected and identified: maltose, galactose, dextrose (or levulose) saccharose, lactose, pentose and inulin.

MALTOSE

Monilia tropicalis	+	= maltose
Monilia macedoniensis	0	

GALACTOSE

Monilia metalondinensis	+	= galactose
Monilia krusei	0	
Monilia macedoniensis	+	
Bacillus morgani	+	= galactose
Monilia krusei	0	
Monilia macedoniensis	+	= galactose
Monilia krusei	0	
Bacillus colombensis (L)	+	
Monilia macedoniensis	+	= galactose
Monilia krusei	0	
Bacillus paratyphosus B	+	
Monilia metalondinensis	+	= galactose
Monilia pinoyi	0	

SACCHAROSE

Monilia tropicalis	+	= saccharose
Monilia metalondinensis	0	
Monilia tropicalis	+	= saccharose
Bacillus paratyphosus B	0	
Monilia tropicalis	+	= saccharose
Bacillus coli-communis, sensu stricto (does not ferment saccharose)	0	

INULIN

Monilia macedoniensis (var. *macedoniensoides*) +
Monilia tropicalis 0
= inulin

LACTOSE

Monilia pseudotropicalis +
Monilia tropicalis 0
= lactose

Monilia tropicalis 0
Bacillus paratyphosus B Schottmüller 0
Bacillus coli-communis or Bacillus coli-communior +
= lactose (with great probability)

DEXTROSE AND LEVULOSE

Monilia krusei +
= dextrose (or levulose)

Chemicomycologic Formulas

SACCHAROSE

Fehling's solution 0
Monilia tropicalis +
= saccharose

LACTOSE

Fehling's solution +
Monilia pseudotropicalis +
Monilia macedoniensis 0
= lactose

Fehling's solution +
Bacillus paratyphosus B 0
Bacillus coli-communis +
= lactose

Fehling's solution +
Bacillus paratyphosus B 0
Bacillus coli-communior +
= lactose

PENTOSE

Fehling's solution +
Monilia tropicalis 0
Bacillus paratyphosus B +
Bacillus coli-communis or *Bacillus coli-communior* +
= pentose (generally arabinose)

Cultivation of the Monilias Kept as Stock Cultures.—The organisms should be grown and kept at 22 C. or at the temperature of the room. Certain monilias, if kept too long in the incubator at 37 C., may lose some of their fermentative characteristics. The best medium is slightly acid dextrose agar. They should be transplanted once a month, and from time to time one should make plates to be certain that the cultures are pure.

RC Castellani, (Sir) Aldo
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